

nature*February 13, 1975*

No longer so much solid ground

A BETTER public understanding of science is an objective being pursued in many parts of the world at present. Committees are constituted to look at the interfaces between science, politicians and the media. Interviewers are despatched to ask the man-in-the-street whether he knows what electricity is, and if so, who told him. Sociologists are busy clipping newspapers and measuring science coverage to the nearest centimeter. And science journalists are being called on to explain why the popular press will run stories on cancer cures in preference to those on radiotelescopes. The driving force for all this activity is undoubtedly a widespread belief that the public is disenchanted with science, and the corollary that an improvement in the quality or quantity of public exposure might result in a better appreciation of the virtues of the pursuit of science.

The very existence of a broadly based public disenchantment must be questioned, the more so since everyone seems to believe in it these days. Ask someone at random what he doesn't like about science and he will probably say "the atomic bomb", or "pollution", or "spending so much money on going to the Moon". After a period of idolisation, scientists have to come to terms with the fact that, like politicians, economists, lawyers and industrialists, they now have to live in a world in which they face frequent criticism about events far too complex to have an identifiable source. This is hardly disenchantment, more an entry into a world in which cynicism is more common than trust, and it is difficult to see that any public relations campaign will succeed, or even that it should be attempted.

There are, however, two kinds of people whose perception of science we ought to worry about: the politician, because he may want to use our results and because he holds the purse strings, and the young student, because he has it in his power to provide or withhold the manpower. To the extent that any politician cares about science at present, a common complaint one hears is that scientists don't seem capable of proffering clear-cut advice. The schoolboy, more forthcoming, may say that science is too complex and offers no obviously profitable pastures these days. Here there is scope for a careful

examination of how the scientist is depicted.

The problem is that, in many minds, a nineteenth century image of the scientist still persists—there are even those within the community who lack sufficient curiosity about their profession to have perceived that the scientist can no longer be portrayed as a bearer of truth. This is not to say that scientists are bearers of lies these days, but it is to assert that the character of most science is now so complex, particularly when dealing with man's interactions with the world, and the purposes for which research is pursued are so varied, that any generalisations that a scientist makes in the literature (and few scientists are happy to let someone else do the generalising for them) are written not on tables of stone, but in chalk, with a good chance of being erased within the year. Scientific statements are falsifiable statements (even though few scientists have read Popper) and in their very nature many of them are indeed going to be falsified.

'Maker of falsifiable statements'—this is hardly a generally understood view of the scientist, and certainly not the kind of know-all image that scientists are often portrayed in the media as possessing.

Yet without stripping away much of the popular mythology surrounding the scientist and his conduct of science, confusion is bound to continue among those who have been led to believe that the scientist's generalisation, having got past a referee or two, is therefore worthy of exceptional respect.

Outsiders and prospective insiders often learn about the true nature of science (which, it must be said, is much more stimulating and exciting than it would be if scientists' pronouncements were only verifiable) by shrewd observation. But it would be better if they could acquire this information more directly. Any project to increase public understanding of science could well devote much of its energy, not to passing round more facts, but to building up, probably through case histories, an appropriate primer on the way in which science knows in part at any particular time—and doesn't always proceed in the most logical way to build on partial knowledge.



Turkey's flower powers

PETER COLLINS REPORTS

THE recent decision of the Turkish government to make trade in opium poppy "straw" a state monopoly is a logical step in the process of assuming complete control of the production and marketing of this crop. It should also reassure those interested people outside Turkey who were surprised, if not distressed, at the announcement some months ago that the ban on growing the opium poppy (*Papaver somniferum*) had been rescinded. In fact, the lifting of the ban, although said originally to have been suggested for political reasons, was almost essential if widespread distress, possibly starvation, were not to be inflicted on the many thousands of farm families for whom this crop provides almost the sole source of income.

The story of this latest phase in poppy cultivation in Turkey goes back to the beginning of the 1970s. At that time, the United States government, acutely aware of the threat of heroin addiction among its younger population, came to regard Turkey as the main source of the opium from which that drug is derived. Thanks largely to the work of the United Nations Narcotics Laboratory in Geneva, it is possible to establish the provenance of opium samples with reasonable accuracy but in any event there is ample other evidence to support this view, although only a small proportion of the world's total opium is grown in Turkey. Determined to deal with the heroin problem, the United States persuaded the Turkish government to prohibit all cultivation of the opium poppy, an announcement made somewhat dramatically at a meeting of the United Nations Commission on Narcotic Drugs in Geneva in 1972. Moreover, it is certainly true that, following the ban, there was a rapid decline in the amount of heroin coming on the market, not only in the United States but also in the Riviera, where there was at one time said to be a "heroin famine".

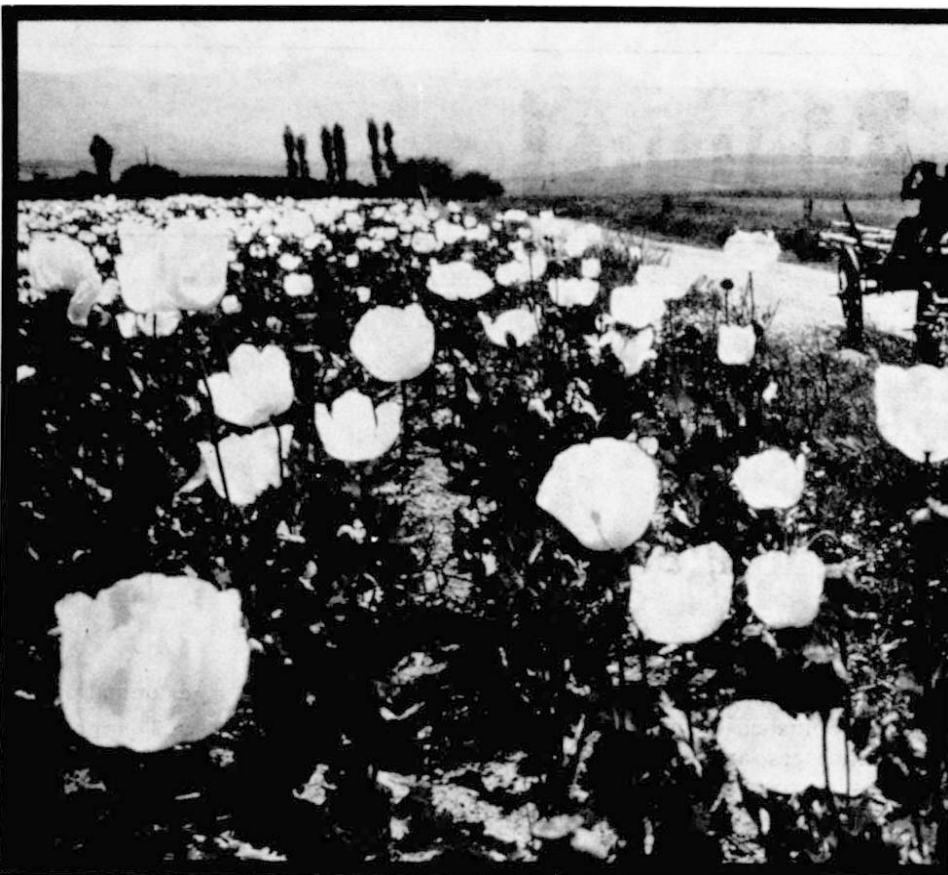
The Turkish ban was imposed on the understanding that the United States would provide the funds—estimated at \$35 million—for recompensing the farmers who were being deprived of their main cash crop, and for the very large research and development programme required to find alternative

crops or other occupations. In the event, while several million dollars were handed out as compensation, the crop-substitution programme never really got off the ground—a factor that may also have influenced the Turks in their change of mind.

The fact is that in those areas of Turkey where the poppy has traditionally been grown, almost no other cash crop will succeed. Poor soils, low rainfall, extremes of heat and cold make normal agriculture difficult, often impossible, over much of those provinces—Afyon, Burdur, Denizli, Isparta—in the south-western part of the Anatolian plateau. To the problems posed by soil and climate were added that natural resistance to change of any agricultural population, especially when that change was apparently being made to please a foreign power for whom in any event they had no particular affection. In fact, the difficulty of substituting new crops, and ideas amounting almost to a new way of life, for opium poppy cultivation has been known ever since the late 1950s when the UN Food and Agriculture Organisation (FAO) became involved in this type of operation in Iran; and the Americans, with their teams of willing but perhaps oversophisticated experts under AID, were even less successful than previous efforts in this direction. It seems almost as though AID was landed with a vast project which it would almost certainly

have rejected had it been suggested to it in the ordinary way of technical assistance.

At this early stage, the United Nations Division of Narcotic Drugs (the secretariat for the Narcotics Commission) was not involved, and indeed by the summer of 1973 it still had little idea of how the American crop-substitution project was progressing, although there were suspicions that things were not moving very rapidly. What seems to have happened next is that the then opposition party in Turkey held out the removal of a ban on poppy cultivation as part of its election programme. Rather unexpectedly, it found itself in power, and committed to implement its promise. Having effectively snubbed the Americans, the party went to the United Nations for advice. With the arrival of a UN mission in the poppy-growing areas, an unappreciated factor became apparent. It was already known that the situation in Turkey was very different from that in Iran, the other Near Eastern country with a major opium poppy problem. In Iran, opium has for centuries been smoked in the villages in the areas where it is grown. In Turkey the villagers do not smoke opium, but rather the importance of the poppy crop lies primarily in the seed, as the principle source of vegetable oil for the local population and, after extraction, of oil-cake for cattle feed. The rest of the plant is





A poppy field in Turkey: Daily Telegraph picture.

been well advanced for some months. Farmers wishing to grow poppies must apply through defined channels, and their applications, with the area it is intended to cultivate, must be duly registered; the total area under this crop has been put at 20,000 hectares (about 45,000 acres) spread over the seven provinces in which it has always been grown. The fact that these poor soils give low yields is of little immediate interest to the government; what matters is that the livelihood of the farmers is assured at least at the previous levels, particularly if the Americans can be persuaded to release the remainder of the \$35 million to help stabilise the situation in the next few years.

It is realised, of course, that this system is unlikely to be completely watertight during the first season, although the UN in Geneva are quietly confident that the Turks will attain their general objective, namely to keep Turkish-grown opium off the world market. The new system will be far simpler and probably cheaper to run than the ceaseless pursuit of illicit opium traffickers, although obviously vigilance will not be relaxed there either. But the government announcement of January 17 makes it quiet clear that the severest measures will be taken against offenders—their harvests will be destroyed, their licences will be cancelled, and no further licence will be granted in the future. Offenders will also be subject to “the relevant provisions of Turkish penal law”.

A system such as this, especially if it is rigorously enforced, should provide the necessary safeguards to reassure the Americans. Recent reactions from the United States show, however, that they remain unconvinced. One Senator has been quoted by the BBC as saying that it was “obvious we will be inundated with heroin” now that the ban on opium poppy cultivation is lifted. This alarmist attitude, as reported by the BBC, betrays a complete lack of faith in the power of the Turkish government to control the situation. It may be that, nettled by the failure of their own huge project, the Americans do not credit the facts about Turkish poppy cultivation reported by the two UN missions. But if the Turkish control system goes as planned, no opium at all will be produced and once the proposed processing plant is in operation, in two or three years time, only drugs such as morphine and codeine will come from Turkey.

Whether American pessimism or Turkish confidence will be justified is perhaps anybody's guess, and will remain so until the critical weeks of the poppy seed—or opium—harvest in May this year. □

normally used for bedding for livestock. This is not to say that no opium was produced, but the poppy heads were lanced and the opium thus obtained sold, principally when a farmer needed extra funds at short notice, as for example for a wedding or a funeral.

The facts on which this UN ‘discovery’ were based had certainly been available to the Americans, since it was in examining their very full statistics that the mission was able to confirm what conversation with farmers and other local people had indicated: that there was little growing of the poppy solely as a source of opium. But the Americans had been so obsessed with the idea that ‘Turkish opium becomes American heroin’ that they had missed the real facts, and gone bull-headed for the complete and immediate prohibition of what was in fact the most important crop for perhaps 100,000 Turkish farmers. Faced with this situation, the Turkish government were to receive some \$35 million for the re-development of the areas affected.

The fact that opium production is not the main objective of Turkish poppy culture added a sound agro- and socio-economic argument to the government's decision to rescind the ban. What will happen now is that the poppy will continue to be grown as before, until the vital two weeks during which the capsules are ripening. It is at this time that, when opium is being produced, the

heads are lanced so that the opium latex can ooze out. It is a laborious business, because the grower must wait 10 to 12 hours before the hardened latex can be collected. Besides keeping several people busy for some hours, this is obviously a difficult process to conceal. It is at this period that an army of government inspectors will descend on the farmers to ensure that no lancing of capsules or illicit sale of materials takes place. Once the capsules have dried, the farmers will be able to collect the seed, both the yield and quality of which is improved when no lancing is carried out. The empty capsules and the upper parts of the stem—the “poppy straw” of commerce—will later be collected by the government for processing to extract the morphine and codeine for the pharmaceutical industry. In this way opium and hence, heroin, is bypassed completely, yet the farmers will still have the rest of the plant for livestock bedding. Moreover, by setting up their own processing plant, the Turks will not only further reduce any chance of their raw materials getting into the illicit market, but will also acquire a lucrative state monopoly. A second UN mission has in fact only recently returned to Geneva after examining the financial and technological aspects of this further project.

On the ground, plans for putting the government's scheme into action have

international news

SINCE energy research and development is a politically popular topic these days, the Ford Administration, which has been searching desperately for something politically popular to announce, has been making much of the fact that massive increases in federal funding for energy technologies were included in last week's budget. The amount has been variously reported as \$1,663 million, \$1,837 million, \$2,115 million, \$2,360 million and \$2,829 million—an array of figures which should further enrich the confusion over energy policy in the United States.

Energy research and development, it should be understood, can be defined in various ways, and various budget figures have been based on different definitions, hence part of the confusion. Another complication is the fact that budget figures are expressed in two different ways—obligations, which represent commitments to pay for grants or contracts although the actual expenditure may not take place immediately, and outlays, which represent the actual amounts of money which each agency will spend in a given year. As far as energy research and development is concerned, there is a considerable difference between total obligations and outlays, all of which makes it a bit difficult to compare what the Ford Administration is proposing to spend next year with what will be spent this year.

Nevertheless, the Office of Management and Budget (OMB), which produced President Ford's budget request, has made an attempt. It has come up with an analysis suggesting that, if Congress agrees to the proposals, obligations for energy research and development will increase from \$1,669 million this year to \$1,837 million next, whereas

Analysing the US energy budget proposals

by Colin Norman, Washington

outlays will increase from \$1,222 million to \$1,663 million over the same period. In addition, obligations for closely related environmental and basic research will increase from \$497 million to \$523 million and outlays on those activities will grow from \$367 million to \$452 million. The total growth in obligations being proposed for direct and related energy research and development is therefore a modest 10%, while the proposed jump in outlays is a spectacular 36%.

It is impossible to tell which individual programmes are being given priority from those figures, however. For that, it is best to turn to a different set of figures—the budget request for the Energy Research and Development Administration, as outlined by Dr Robert Seamans, ERDA's Administrator, to the House Committee on Science and Technology last week. Since ERDA is now responsible for the bulk of the Federal government's energy research and development, the ups and downs of the budgetary fortunes of its various divisions should provide a measure of which energy programmes are being favoured.

Seamans presented the committee last week with a table (see below) which provides details of the agency's budget request for next year. The table is expressed in terms of outlays, and although the total is greater than the

amount shown in the OMB's analysis for the entire federal energy research and development budget (the difference, an ERDA official supposed, probably arises because different things have been included in the summations) it provides further evidence of the dominance of nuclear power in the Administration's long term energy strategy.

Between them, reactor research and development, nuclear materials production and advanced isotope separation technologies—chiefly centrifuge enrichment—are set to receive \$1,118 million next year, which is an increase of \$236 million. Moreover, more than \$400 million is budgeted for capital expenditure on fission technologies, so the nuclear energy programme is again set to carry off more than half of the total energy research and development budget. The fact is unlikely to be accepted by Congress without demur, since there is a groundswell of opinion building up on Capitol Hill that non-nuclear technologies have been relatively neglected.

Although the figures proposed for such items as solar energy, geothermal power, and fusion look generous enough, the actual amounts indicated in ERDA's budget request are unlikely to satisfy most supporters of those programmes. The solar energy budget, for example, which is set to increase in ERDA from \$8.8 million this year to more than \$57 million next, should be seen in relation to Congressional appropriations for 1975 of \$50 million. Furthermore, a panel of experts told the Atomic Energy Commission two years ago that a minimum acceptable programme for solar energy development would involve expenditures of at least \$65 million in the 1976 fiscal year. Similarly, Congress authorised expenditures on geothermal energy in 1975 amounting to some \$44 million, yet the expenditure being proposed for ERDA in 1976 amounts to only just over half that. Although the National Science Foundation still has some programmes involving basic research related to solar and geothermal energy, Congress is unlikely to be satisfied with the amounts being proposed for next year in those areas.

It should also be noted that President Ford has formally proposed to reduce the amounts appropriated by Congress for ERDA in 1975 by some \$85 million; that proposal is reflected in the figures present in his budget. If Congress refuses to go along with that request,

ENERGY DEVELOPMENT PROGRAMMES	1975*	1976*	Change	
		\$ million		
Fossil energy development	195.0	311.3	116.3	59.6
Fusion power research and development	85.0	120.0	35.0	41.2
Fission power research and development	384.1	443.7	59.6	15.5
Nuclear materials production (less weapons activities)	445.2	596.1	150.9	33.9
Advanced isotope separation technology and laser fusion	53.1	78.2	25.1	47.3
Solar, geothermal and advanced energy systems development	34.9	108.6	73.7	211.2
Conservation research and development	16.7	32.2	15.5	92.8
Physical research	281.6	312.5	30.9	11.0
Environmental and safety research	165.0	197.7	32.7	19.8
Plant and capital equipment for above	553.4	629.2	75.8	13.7
	2,214.0	2,829.5	615.5	27.8

*Fiscal years.

which is likely, then the totals shown in the table for 1975 would increase, the totals proposed for 1976 would decrease by a similar amount, and the total increase being proposed for the agency would shrink considerably.

In any event, Congress is likely to reorder the priorities in ERDA's budget a little, certainly by increasing non-nuclear appropriations and probably by reducing the amounts proposed for fission technology as well.

Even according to the OMB's analysis, however, the total amount of money that President Ford has proposed to spend on energy research and development in 1976 is more than double the total spent in 1974—\$1,663 million compared with \$833 million. A question that has not been raised too openly is whether that huge surge in funds can be usefully absorbed in such a short space of time. □

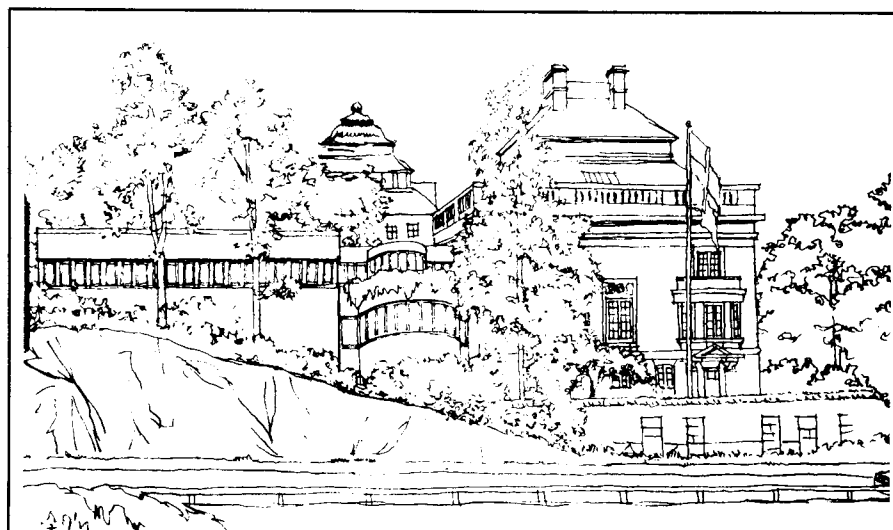
Mining company claims sea bed

from Peter J. Smith

THOSE who see exploitation of the ocean floor as a potential source of world conflict will take little comfort from a paid advertisement which appeared last week in *The Times*. For in the small-print Public Notices column, Deepsea Ventures Inc., of Delaware and Virginia, announced that "it has discovered and taken possession of . . . a deposit of North-east Pacific Ocean seabed manganese nodules."

The section of the sea bed over which the company claims "exclusive mining rights" is the area bounded by the latitudes 14° 16'N and 15° 44'N and longitudes 124° 20'W and 127° 46'W—a region lying in international waters between the Hawaiian Islands and mainland North America. Within this zone Deepsea Ventures intends to "develop, evaluate and mine the deposit and to take, use, and sell all of the manganese nodules . . . and the minerals and metals derived therefrom". As a form of "diplomatic protection and protection of investment" the company also "requests and requires [*sic*] states, persons, and all other commercial or political entities to respect the exclusive rights asserted".

Deepsea Ventures says that the validity of its claim is established "under existing international law as evidenced by the practice of States, the 1958 Convention on the High Seas, and the general rules of law recognised by civilised nations". In fact, the legal position is far less certain than that statement would suggest; the 1958 convention, for example, made no specific mention of any freedom to exploit the deep sea bed because at that time



Not all of Pugwash's cries fall on deaf ears. A little known Swedish listener has offered to give substance to one of Pugwash's recent pleas: for an international institute to carry out advanced theoretical scientific studies into energy and ecology problems.

The pricked ears belong to Kjell Beijer, a Swedish businessman who last year set up the Kjell and Märta Beijer Foundation for the advancement of scientific research in Sweden. The foundation has decided that its first project will be the financing of the new institute, and is to donate Sk12 million (about \$3 million) over an initial period of 10 years for running costs and SK3 million for the construction of a building—to be attached to the Royal Swedish Academy of Sciences—in which the institute will be housed. The building should be ready by the end of 1976.

The institute will have close links with the academy in more ways than one. The academy is to be responsible for its establishment, and is at present working out the details of its organisation with the Royal Swedish Academy of Engineering Sciences. The main idea is clear: the institute should be shaped along the same lines as the Stockholm International Peace Research Institute (SIPRI). An international board of scientists will be responsible for the overall planning of the work, and a director (not yet appointed) will run day-to-day activities, make suggestions to the board about new projects and carry on his own research. The actual projects will

Architect's sketch of the Royal Swedish Academy of Sciences building showing how it will look when the new institute is attached (at left).

be carried out by 10–15 scientists, including guest researchers who will be invited to work on problems in specific areas. Four areas have been suggested so far:

- Surveys of energy resources, including new primary sources, such as solar, geothermal and fusion energy.
- Energy and economic development, taking into account the energy problems of developing countries.
- Energy and environmental effects, including the protection of the atmosphere against thermal, chemical and nuclear damage.
- Energy in relation to other resources, for example, minerals, water and food.

The focus will be purely theoretical. No experimental work is planned. This follows from the conception of the institute as a collector, analyser and disseminator of information to governments and the growing number of groups around the world who have started to take an interest in energy questions. The most visible of the institute's activities will be conferences, workshops, symposia and publications.

And when will work begin? With luck before the building itself is ready. But even if it takes until 1977 to work up full steam, the scientists can be sure that there will still be lots of energy problems left for them to solve.

Wendy Barnaby

exploitation was considered impractical.

In effect there is no international law on this matter—an omission which gives equally eminent authorities the opportunity to disagree about whether or not any form of occupation of the ocean floor is allowed. Last year's Law

of the Sea Conference in Caracas failed to resolve the question. It rather looks as though Deepsea Ventures has timed its announcement to concentrate the minds of the delegates when the conference reconvenes in Geneva next month. □

THE congress of ANZAAS—the Australian and New Zealand Association for the Advancement of Science—drew a crowd of 2,500 to the Australian National University campus in Canberra last month. Not as good a crowd as at some previous gatherings said the old-timers, and the spread-out facilities led to a feeling at times that we were the only ones there. Nonetheless, such an attendance was huge by British Association standards.

Many scientists find this sort of forum difficult to handle. For one thing there is uncertainty about who comprises the audience. For another, why launch the latest research result to a dimly discerning audience in Canberra when you can fly to London, Moscow or San Francisco to tell the specialists? This undoubtedly largely accounts for the relative poverty of the physical science section, but other sections gain by their very breadth of appeal. History, geography, education, industrial relations and economics all find a home in ANZAAS and, it is said, grow. But food science and nutrition attracted most attention this year. This could be seen partly as a reaction to the world food situation and partly as a move towards whole-animal (and especially whole-man) science, but maybe also as a reflection of an uneasiness amongst Australians that their diet encourages obesity. And all the signs are that obesity is on the increase.

The theme for the conference, "Science, Government and the People" was largely pursued through nine symposia inconveniently scheduled so that nobody could attend more than three. The quality was very uneven, but probably those who attended diligently emerged with a broader understanding of the scientific community and its external interactions. A national project for the public understanding of science 'warts and all' was one practical proposal that emerged.

It was encouraging that Mr Bill Morrison, the Minister for Science, and Mr Tony Street, Opposition spokesman, were so much in evidence and participated actively in the proceedings. Would that a few politicians in other countries made themselves visible and accessible in such gatherings, if only because most scientists view science policy as something remote and irrelevant and could profitably learn what the politicians think about it.

● Police vans flanked the entrance to the Academy of Science for the symposium on scientific and technological research in universities and colleges of advanced education. Hardly an explosive subject, but the presence of Professor Roger Russell (Vice-Chancellor of Flinders University) guarantees a demonstration these days. Professor

Russell worked in the United States on various projects related to psychological warfare for the Department of Defense and according to some he still has involvements which they want him to disclaim publicly. There appeared to be about 20 in the audience of 200 who came to heckle and nearly twenty television technicians whose intrusive role in triggering confrontation was fairly obvious; but after some tough-mindedness from the chairman, Professor J. M. Swan, resulting in the ejection of four demonstrators, the

Australian diary

from David Davies
and Peter Pockley



Fly me

meeting was uneventful apart from some fairly conventional interruptions. Maybe three and a half hours on the binary system in advanced education was more than any demonstrator could bear. Maybe they feared for the safety of their tape recorders if ejected. Or maybe it was the fear of being dumped in the moat surrounding the academy.

● With the exception of the Pharmaceutical Sciences Section which regularly brings a team of overseas experts to Australia for these congresses through direct funding by drug companies, ANZAAS meetings seldom attract contributors from overseas to present papers of international significance. It was therefore all the more notable that Dr R. J. Murgatroyd, Chairman of the British Meteorological Office's Committee on the Meteorological Effects of Stratospheric Aircraft (COMESA), chose ANZAAS as the venue for a major announcement.

Australia has been the scene of extensive public debate on the alleged deleterious effects of the SST aircraft, particularly Concorde. Various airlines want to fly it to Australia from Europe and America (the London-Sydney trip could be cut by half to 12 hours

or so). An environmental bandwagon against Concorde has had considerable political effect over the past three years. Despite an Academy of Science report which cautiously cast doubt on the validity of theoretical projections by American and Swedish chemists, the Australian government has so delayed confirming orders for Concorde that most people think the deal is off.

Dr Murgatroyd's conclusions were clear—several hundred Concordes would not reduce the stratospheric ozone concentration by more than 1%, 1,000 Concordes would cause a reduction of about 3%, and 2,000 Concordes about 6%. Because of their higher operating altitude (20 km, as against 17 km for Concorde) and their higher engine emissions by virtue of a greater size, American-designed SSTs could produce roughly twice the effect. Climatic changes brought about by Concorde would similarly be minute, several hundred Concordes resulting in a variation of less than 0.1 °C. Formation of condensation trails would be unlikely to lead to anything more than minor increases in stratospheric clouds, and these only in equatorial regions and polar areas in winter. All effects are well within natural variations (for example stratospheric ozone has been found to vary by $\pm 6\%$), and the effects of subsonic aircraft may be greater in some cases.

The conclusions of COMESA according to Dr Murgatroyd, are consistent with the results of parallel American and French government research programmes, the American research having now been published as well. The greatly increased confidence of their predictions is accounted for by several factors, all linked with the availability of massive and reliable data on natural atmospheric processes, engine emissions, numbers of aircraft, flight routes, photochemical reactions and mathematical modelling.

Dr Murgatroyd considers the ozone in the stratosphere to be under greater threat from the possible deleterious effects of freon gases from pressure pack sprays and refrigeration equipment. First rough calculations suggest a reduction of 1% in O₃ at present levels, but in 15 years the reduction could go up to 5%.

● The campaign against Concorde and the fears about damage to the Great Barrier Reef by *Acanthaster planci*, the so-called Crown-of Thorns starfish, rank equally as the biggest single environmental issues raised in Australia. Over the past five years or so, the apparent southward march of the starfish along the 2,000 kilometres or so of the largest string of coral reefs in the world has retained the interest of public and politician alike. The campaign for direct action to "save the

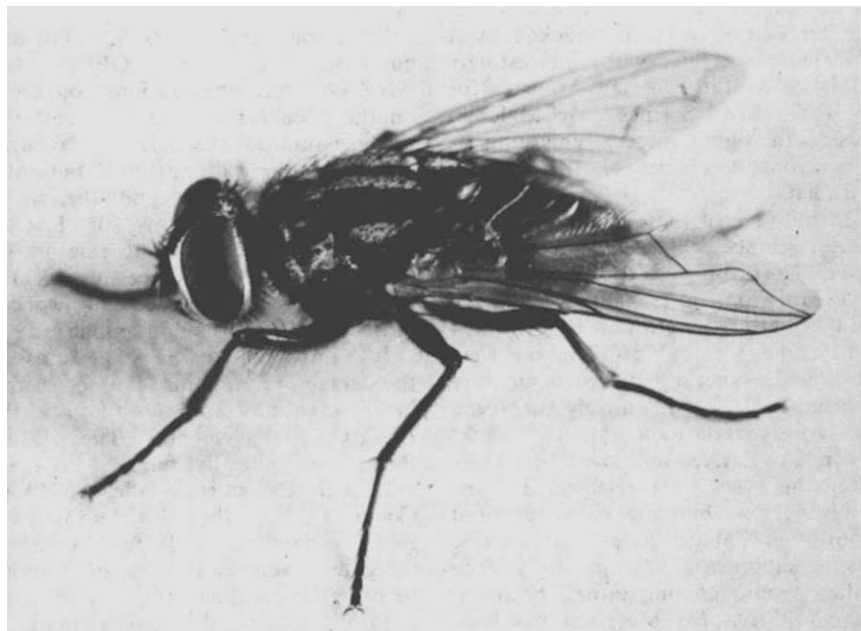
reef" has been led by some zoologists in Queensland, and although nobody has doubted the existence of a real problem, there has been considerable disquiet among less vocal scientists regarding some proposed solutions (for example, direct killing of starfish *in situ*, using teams of divers) and the emotionalism which crept into the publicity.

Nonetheless, the Australian government established a special Advisory Committee for Research on the Crown-of-Thorns Starfish to administer a research fund. \$A330,000 (Aust) was allocated in the first three years of the committee's existence (1972-4), and the original limited programme has been extended for a further year with \$150,000. More government support went into an ambitious survey of 25 reefs in the Great Barrier Reef geological province last year, using ships and divers from the Royal Australian Navy. Mr Richard Kenchington, research fellow of James Cook University, Townsville, who co-ordinated the survey, reported at the ANZAAS Congress that the numbers of starfish on 14 of these reefs were negligible. This, however, has not led to complacency, for the threat to research areas covering the whole range of marine science, such as that at Heron Island, and the economically important tourist reefs is still keenly felt.

Mr R. G. Pearson, of the Department of Primary Industry, Queensland, reported some encouraging results on his surveys of the rate of regeneration of coral reefs after the 'wave' of starfish has passed. Soft corals appear to re-establish themselves in a few years. This kind of research and that concentrating on the largely unknown biology and behaviour of the species were strongly commended for future starfish research by a panel of influential scientists at ANZAAS.

Among other hard-hitting speakers, Dr Frank Talbot, Director of the Australian Museum, Sydney, said the starfish "plague" is not a real problem, is not man-induced and should not be supported by a special research fund. Others welcomed the strategic significance of the public support for the starfish research programme in that it focussed attention in Australia on the long neglected marine sciences and on the great ignorance of the Great Barrier Reef. Now that the ambitious Australian Institute of Marine Science is under way, the general consensus of speakers seemed to be that the days of a research programme relating to a single species and absorbing a substantial proportion of marine research funds should be numbered.

● The announcement at the congress of



THE most recent British Airways advertisements for Australia show a gnarled man (opposite) wearing a hat around the brim of which dangle a dozen corks on the end of pieces of string. The purpose of this hat has been widely debated in Britain, and the British visitor will find even more mystification when he lands at Canberra to be greeted by what appear to be royal waves from the locals. The explanation of both these phenomena is the ubiquitous bush fly, *Musca vetustissima*, and no tour of the capital is complete without a visit to the entomology division of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) to find the latest on biological control of this pest.

Dr D. F. Waterhouse, director of the division, wrote in *Nature* in 1973 (246, 269) about the dung problem in Australia. Thirty million cattle each produce twelve dung pads a day, and the means of their disposal are on the whole lacking in Australia. Whereas in other countries various dung beetles get to work in their tunnelling and ball-rolling as soon as the dung hits the ground, in Australia it can sit there for years, eclipsing signi-

ficant areas of land, leading to grass that cattle won't eat and providing an ideal breeding ground for flies.

Gradually the problem is yielding. Sterilised imported dung beetle eggs from Africa have been used for several years to generate huge populations of coprids which have been spread around northern Australia with some success. It has become clear, however, that it is necessary to supply a variety of species (in Africa there are at least 2,000) if the attack is to be entirely successful.

And will the farmer accept an intrusion of scientists on his cow patch? Yes, says Dr Waterhouse, if things are done through the proper state channels.

Not all the division's work is unmitigated dung dispersal. There are new and highly successful techniques for eliminating insects from grain storage without resort to malathion. There are attacks on the skeleton weed by the use of fungi. There are genetic assaults on the sheep blowfly. There are even new ideas for fruit tree pruning. The returns on investment are potentially high as some pests cause tens of millions of dollars worth of damage every year.

the terms of reference for the Australian Science and Technology Council (ASTEC) does not guarantee the rapid formulation of Australia's first clearly enunciated science policy. The main danger to ASTEC is precisely that which afflicted the defunct Advisory Committee on Science and Technology, appointed by the last Liberal Government in 1972: the threat of imminent political change.

Since Mr Whitlam's Labour Party

was re-elected in May 1974, the government has lost its public support to a quite remarkable degree. Inflation hit Australia hard only in the past eight months and is now running about 20% a year. Unemployment has risen to a near-Depression level of almost 4%, an intolerable situation for a socially oriented Party. There is daily talk about whether the Opposition-dominated Senate will force another election this year, as they did last year

by refusing to pass the government's money supply plans. On the other hand, the Opposition itself is wracked by a leadership struggle, with, interestingly enough, the Liberals' last Minister for Education and Science, Mr Malcolm Fraser (a right winger), emerging as the strongest challenger to Mr Bill Snedden.

In the face of these running political issues, science still seems pretty small beer. The Minister for Science, Mr Bill Morrison, just made it into the ministry after the election in May 1974—he came 27th out of 27 in the elections to Cabinet by his parliamentary colleagues. It is not entirely surprising therefore that it took until two weeks before ANZAAS, and over two years after he was first elected to the ministry, for him to gain sufficient priority in Cabinet to present his first major submission relating to science policy. Some commentators, however, have felt that Mr Morrison has been content to let things drift for, apart from the consumer cause which he has espoused with some energy, he has seen little political advantage to be gained in science.

The two-year wait has been a frustrating one for those Australian scientists who had been looking to Labour for action. Labour had a science policy in its Party Platform for some time in contrast to the Liberals' steadfast refusal to acknowledge the very possibility of, or need for, a coordinated approach to science. The year 1974, however, brought forth a rash of documents and discussions which have led to the final formulation of ASTEC, and no interested party can now complain that their voice has not been heard. There are some, though, whose voice may have been heard but not heeded. Notable in this category must be the Department of Science whose heads can hardly be happy with the statements in the White Paper launching ASTEC which show that the department's role in recommending policy to the government will be subordinate to that of ASTEC.

Mr Morrison's major move towards gaining a consensus about a science council was the invitation to the OECD to carry out one of its independent surveys of science policy. This study, under the leadership of the redoubtable Dr Alexander King, was carried out early in 1974 and led to a preliminary report by the OECD and one of their "confrontation" meetings in Paris last autumn. Meanwhile, ANZAAS had established a Science Policy Commission under the chairmanship of Professor Sol Encel, a sociologist at the University of New South Wales, who is one of Australia's few professional students of science policy. Its report was published in the November

1974 issue of ANZAAS's journal, *Search*.

With some differences in detail and emphasis, both the OECD and ANZAAS recommendations converged on the need for a council to embrace not just traditional science, as favoured in the earlier discussions, but also technology, medicine and the social sciences. The Academy of Science also played an important role in influencing the final shape of ASTEC but, as is its wont, the academy worked more in private than in public.

It is impossible at this stage to assess the degree of acceptance by the Australian scientific community of the ASTEC arrangements. The White Paper was officially released to coincide with the start of the ANZAAS Congress, but the distribution was ineptly handled and few congress delegates even had sight of a copy before the major symposia at the congress where the issues involved were discussed. A meeting to discuss the ANZAAS report mustered a mere 70 in the audience.

But it is already clear that Mr Morrison's proposals (he claims to have largely written the White Paper himself with help from his staff—there is no evident love affair between the minister and his public servants) comprise a minor political victory in three areas, all of which will be welcomed by Australian scientists. These involve the terms of reference and powers of ASTEC to encompass not only science, but also technology, the medical sciences and defence science (within the limits of security). To the disappointment of some, ASTEC's role in relation to the social sciences is not at present intended to extend beyond the interaction of the social and natural sciences, as in multidisciplinary studies of complex problems.

The political victory comes in that funding of research and development technology, medicine and defence have come exclusively under ministries other than Science, and this has contributed substantially to the fragmentation and lack of direction in Australia's overall science effort. Proposals for the integration of these disparate elements will have to be formulated with great

bureaucratic and political skill by the members of ASTEC, and therein will lie a major test of its potential influence in the science scene in Australia.

The Labour Party's Platform had advocated that the members of a science council be elected by the scientific community; this has been abandoned in favour of the traditional method—appointment by the Minister, who significantly for the status of ASTEC will formally be the Prime Minister. ASTEC will be unusually sensitive to the quality of its first appointees, and further significant commentary on it may have to await that announcement.

● Gough Whitlam himself gave a paper at the 'Grand Symposium' on the final night. Recently returned from extensive foreign travels, he shared the platform with the Presidents of the Academies of Science, Social Sciences and the Humanities in an evening of remarkably good public speaking. His speech was mainly directed at the rationale behind the science policy White Paper, but he surprised the audience by taking the floor again after the main speeches to deal firmly with issues raised—particularly some questioning of his uranium policy and some remarks about trying to keep academics, particularly in the humanities, from emigrating. Professor J. Passmore (Academy of the Humanities) had been bemoaning the poor quality of Australian libraries and the great delays in the arrival of journals.

Nowhere on his travels, Whitlam said, had anyone suggested that the uranium should be kept in the ground, although he was fully aware of proliferation problems and hoped to "do something" about them.

On emigration, he professed no concern. Maybe the scholars move away but the practitioners come back to play, sing, paint and so on. Not quite the same thing, muttered the audience as they left, dazed that even a mild bit of politicking had entered their sedate arena. And there wasn't a glimmer of a response to Passmore's gentle suggestion that the government might air-freight journals from Europe and North America. □

Must new universities be poor relatives?

● In 1946 there were 25,600 students in six Australian universities. In 1972 there were 128,000 in eighteen. But there is a failure rate of 30% and many academics speak with concern of the quality of staff in some of the newer universities. If these universities are not to be seen as poor relatives, they face a long haul of consolidation. There

could be a major role for CSIRO in this. The OECD report speaks of a need for greater mobility amongst Australian scientists and it may be that the new universities, armed with this report, should seek strengthening of their staff through more direct collaboration and exchange with CSIRO.

A searching look at Israeli science

from Nechemia Meyers

AN extraordinarily incisive and explosive 71-page report on the interaction between Israeli industry, science, universities and government was released this week by the Jerusalem-based National Council for Research and Development after being kept under wraps for two years. The report, submitted to the National Council for Research and Development (NCRD) in December 1972 by Dr S. Wald, a well known OECD economist and expert on science policy, notes that the country invests very substantial amounts in research and development (2.5% of its GNP) and that its research is of very high quality. Nevertheless, Wald charges, Israel's policy for research and development lags behind that of western Europe and North America "perhaps more than it did 20 years ago".

The factor primarily responsible for this situation, in Dr Wald's opinion, is an insufficient emphasis on applied research. "Israel," he says, "appears to be the last bulwark of the old faith which puts theory and pure science above practice and applications".

Wald caustically suggests that Israeli professors, to whom he attributes great influence, "may have greater respect for discovery than for development because few have had experience in development work and thus can hardly be expected to realise that the discovery of new material is sometimes intellectually less demanding than the discovery of methods to produce that material in tons and for an acceptable price".

Government ministries, Wald argues, have little more understanding of the situation. In the words of the report: "they act as if once a great 'discovery' is made its 'development' will be routine work and can more or less take care of itself".

Even industrial companies "have interiorised the prejudices of which they are the main victims", Dr Wald believes, adding that men who in Europe would be called "research directors" and in the USA "vice-presidents for research" are in Israel termed "development directors". This suggests that "nothing or almost nothing of their work would qualify as 'real' research".

Dr Wald reports on conversations with Israelis who link the country's single-minded dedication to pure research with Jewry's traditional emphasis on learning for its own sake. The image of Einstein, who changed the world and gained immortality with the stroke of his pen, was also invoked. This image,

Wald says, "has perhaps left a more disastrous mark on the minds of young people in Israel than in any other country".

Credit must certainly be given to the NCRD, which is part of the Prime Minister's Office, for publishing a document which declares: "No government in the Western World has so much concentrated economic and industrial power, yet none uses it so little to define and to achieve precise technological goals involving industry and universities".

It is perhaps not without significance that the document, which takes the government to task for failing to deal with questions of science and technology on the Cabinet level, was released only after the belated appointment (last month) of a Ministerial Committee on Science and Technology.

One goal of this new committee will certainly be to coordinate existing research programmes of government ministries which, Wald says, "to some extent still consider each other as competitors and not as complementary instruments of one and the same nation". His report cites the example of a project which the Ministry of Commerce and Industry approved on scientific grounds, but would not finance because telecommunications "belonged" to the Ministry of Posts and Communications (which at that moment had no applied research fund).

Marketing is much underrated in Israel according to Wald, who found it regarded "as a second-rate propaganda job", rather than as a suitable occupation for skilled scientists and engineers. The OECD economist also questions the Israeli tendency to look for large American corporations to serve as partners and marketing agencies for local industry. These big companies, he warns, are likely to view Israelis as very minor partners who can be dropped quickly if other considerations arise (a need to cultivate the Arabs, for example).

Dr Wald suggests that Israel should model her technological development programmes on those of small Western European countries like Holland, Switzerland and Denmark rather than on the USA. He points out that, like Israel, they have meagre natural resources and that their relative prosperity has been achieved by concentrating on the development of a few quality products, with which they are capable of competing on the world market. Israel's key products, he says, might come from the electronics and aeronautics industries, and more particularly from a few companies, like Israel Aircraft Industries, with sufficient turnover to finance a serious programme of research and development.

Western European countries accord

applied research and engineering higher prestige than they enjoy in Israel, Wald notes, mentioning in this context the fact that these nations sometimes create highly prestigious national academies of engineering science, a parallel to their science academies. Moreover, their universities are quick to meet local industrial requirements. When the Swiss watch industry was in trouble, Wald recalls, the university in Neuchâtel set up a faculty to train watch-making engineers "without worrying whether watch engineering was a recognised engineering profession, was a 'science' that could lead to publications or was represented at MIT".

Wald notes that Israeli professors have in recent years stressed their commitment to industrial development, but wonders if they are indeed prepared for a drastic change in science policy. The fact that Israeli industrialists still receive letters of recommendation from professors stating that a particular student is not quite good enough for PhD work but would make an excellent researcher in industry "speaks louder than public declarations", Dr Wald says.

With all his criticism, the OECD economist remains optimistic about Israeli's industrial future. "A visitor", Wald observes, "has the almost daily impression of an exceptional reservoir of will and talent which could be organised with more efficiency and profit. Many of the ingredients which make for a powerful industry-technology-science interface can be found in the country. Like pieces of a puzzle, they lay there—some fit together already, others are still in disparate order awaiting the hand that will push them together". □

First catch your bear

THE Soviet Ministry of Agriculture has drafted a special programme, within the framework of the existing international agreement, for the protection of Polar Bears. Accordingly, an expedition is shortly to leave for Wrangel Island, which has the largest bear colony in the Soviet Arctic.

The tagging of adult bears and their cubs is to take place in March and April, when the bears bring their cubs out to bask in the sun. The procedure, it is announced, will take the form of painting a number in red on the backs of the bears and the insertion of metal rings in their ears.

The *Novosti* press release announcing this expedition is headed optimistically "Polar Bears no longer threatened with extinction". A happy prospect for the bears—but perhaps less so for the painters and ear-ring fitters. One hopes that the expedition will be equipped with a supply of stun-guns. □

correspondence

Czech mathematician

SIR,—On January 10, 1975, a petition was submitted to the Czech embassy in the Netherlands, concerning the position of the Czech mathematician and computer scientist Professor Karel Culik. The petition was worded as follows:

"The undersigned mathematicians and computer scientists are deeply concerned with the present position of Professor Karel Culik. Although his work has done much for the world-wide renown of Czech mathematics he is at the present time prevented from taking part in normal scientific activity; he has no position in his own country and at the same time cannot obtain permission to accept the offers he has from universities in foreign countries. The undersigned strongly urge the Czech authorities that Karel Culik be allowed shortly to pursue his career as a researcher and a scientist."

The petition was initiated by the European Association for Theoretical Computer Science, and signed by 238 scientists from 12 countries.

Yours faithfully,

J. W. DE BAKKER

(Vice-President, European
Association for Theoretical
Computer Science),
Amsterdam, Netherlands

Consultancy

SIR,—Two correspondents (December 13 and January 17) have recently criticised the way in which university staff misuse the facilities at their disposal by carrying out consultancy work.

There are other ways in which university staff take advantage of their position, particularly their flexible hours of work. Many for example mark GCE examination papers, or act as GCE examination supervisors, or as tutors at Open University Summer Schools. For these duties they are either paid or receive generous expenses.

Yours faithfully,

DERRICK BAXBY

University of Liverpool, UK

Golden handshake

SIR,—You make the proposal (January 10) that the golden handshake is the solution to the job stagnation problem in British universities. As a younger lecturer (holding an appointment in dental biochemistry) I feel such a measure would only make the situation worse. Senior experienced staff

would be removed and this would only add to the administrative duties and frustration of younger colleagues at the most intellectually fertile period of their careers.

An alternative solution to the problem would be the introduction of opportunities for lecturers who wish to retrain for other professions in the middle of their careers, with financial assistance during this period of training comparable to their existing salaries. Those in their late thirties still have 60 to 70% of their active careers before them.

The people concerned are first-rate men and women, who have reached their present positions in a highly competitive and selective field. Prior experience in academic life could make an important contribution to a subsequent career in a different profession. For example, the experience and knowledge gained by a biochemistry lecturer would be an invaluable asset to someone subsequently studying medicine.

Mid-course retraining is already practised in industry; it could well offer the solution to the academics' problems too.

JOSIE A. BEELEY

University of Glasgow, UK

For those in peril

SIR,—Your correspondents in this series (October 18, November 22, November 29) do not distinguish clearly between the scientific and political aspects of pollution. Peter J. Smith's statement that "asbestos products (and thousands of other equally or more dangerous substances) are here to stay" is not a law of nature, it is a political statement. It indicates that we are prepared to pay, wittingly or unwittingly, with our own, or other people's tissues for the goods and services that these substances offer.

To be able to say under what conditions they are to stay, the scientists will have to tell us about the medical effects of existing pollutants in industrial environments and in the general environment, whether the effects are dose-related, how concentrations can be monitored, and how potential pollutants can be recognised—all difficult, expensive and time consuming problems. But the medical scientist cannot judge this—to him all avoidable tissue damage is totally abhorrent. 'Public opinion' has to decide how much discomfort or loss of years an individual

must pay.

How can the cost or risk for each pollutant be presented intelligibly for the public to judge its acceptability? Setting out the expected health effects, as for SO₂ and smoke, is one way. But concentration-time scales would incorporate commercial and political realities. LD₁₀₀ (concentration X for N years) is bad for business (and votes). A lower product for ill-health may be commercially acceptable in terms of lost man hours or compensation, but becomes less acceptable in the context of individual workers.

But the data must be sound. Unsound scientific or political activism could so limit industrial or daily activity that ill-health from pollutants could become replaced by poverty.

W. F. WHIMSTER

London SE3, UK

The economics of recycle

SIR,—In order to put recycle in a proper perspective (January 17), it is necessary to remember the following factors:

The future availability and price of key raw materials, as well as of oil, have been placed in doubt by the events of the past year. These events have emphasised the need to conserve our national resources. Recycle, or reuse of materials, is an important aspect of the proper management of these resources. It is, however, but one aspect and should be operating along with other measures such as improved design and reduction in waste both in manufacture and use.

Recycle is only viable for those materials which can be collected, reprocessed and made into products which can be sold at competitive prices. The problems are therefore basically technical and economic.

T. S. McROBERTS

Wolfson Recycle Unit,
Queen Mary College, London, UK

Nutrition

SIR,—I would be grateful if you would make it clear that the views expressed by Mr John Rivers in your article entitled "Between combine harvester and ribosome" (January 10) is an expression of his own views. The article should not be regarded as an official pronouncement of this institute.

L. G. GOODWIN

Nuffield Institute
of Comparative Medicine,
Zoological Society of London, UK

news and views

Molecular evolution and the age of man

from John Maynard Smith

It is always intriguing when scientists with different backgrounds and training apply their different techniques to the same problem, and come up with totally different answers. This situation has now arisen in the study of human evolution. The traditional palaeontological approach suggests that the lines leading to man and to the great apes diverged at least 15 million years ago, and perhaps longer; biochemical evidence has recently been interpreted as showing that the time could not have been more than 5 million years.

At first sight, a comparison of proteins of different species seems just one more phenotypic comparison providing information similar in kind to that provided by a comparison of bones or teeth. This is not so. Knowing that the primary sequence of, for example, the insulin A chain in guinea pig and coypu differ by six amino acids, we can assert that at least six gene substitutions must have occurred in the relevant gene along the lineages leading from the common ancestor to the two species. A knowledge of the genetic code tells us that two of these substitutions could not have taken place in a single step, and hence that at least eight substitutions have taken place. Of course, these are minimum estimates. If the same changes have taken place in both lineages, or if some sites have changed several times, we shall underestimate the actual number of changes. This difficulty is a real one, but is probably less serious than might have been expected. A comparison of two proteins does enable us to estimate the number of gene changes that have occurred; we have no idea, even to an order of magnitude, of the number of gene changes responsible for the difference between the canine teeth of ape and man.

This method, combined with absolute dating from the fossil record, has led to some generalisations. Different types of protein evolve at very different rates. For example, cytochrome C evolves rather slowly, and fibrinopeptides (small peptides cleaved off fibrinogen when clotting occurs) very fast. A given class of proteins, however, evolves at a rather constant rate in different lineages. The exact degree of uniformity has been hotly debated, partly because of its relevance to the

'neutral' versus the 'selective' interpretation of protein evolution. The neutral theory asserts that most (though of course not all) amino acid substitutions which occur in evolution are selectively neutral. It is a prediction of this theory that the rate of evolution of a given class of protein should be uniform; hence the heat. Some striking exceptions are known; for example, insulin is remarkably uniform among mammals, except among hystricomorphs (a group of rodents which includes the guinea pig and coypu), which differ from each other and from other mammals by ten or more substitutions. A number of less striking discrepancies are known, but one is left with the impression of a surprising degree of uniformity of rate, at least when compared to morphological characteristics.

Unfortunately, it takes a lot longer to sequence a protein than to measure a bone (and longer still to discover the tertiary structure of a protein, which is a necessary first step if one is to make functional sense of the amino acid substitutions). This difficulty can be partly overcome by use of immunological techniques. A protein from species A is purified, and antiserum to it prepared. The 'microcomplement fixation' technique is then used to compare the extent of the reaction between the antiserum and the original protein from species A with the reaction with proteins from other species B, C, D and so on; it is a major convenience that the latter do not need to be purified. In this way, the 'immunological distance' between proteins can be measured. The technique has been extensively used in evolutionary studies by Sarich, Wilson and their colleagues. The protein used has been albumin; this is a large protein which evolves rather rapidly and so provides a lot of information. There is evidence, for albumin itself and for other proteins, that immunological distance measured in this way is linearly related to the number of amino acid substitutions. The relation is not exact, but it is close enough to make immunological distance a reliable guide to number of genetic changes.

The rate of evolution of albumin in different groups of vertebrates is apparently rather uniform (very approxi-

mately, two amino acid substitutions per million years, in a peptide 580 residues in length). As mentioned at the beginning of this article, the most controversial finding (Sarich and Wilson, *Science*, **179**, 1146; 1973) concerns the evolution of man. Three lines of biochemical evidence (immunological distance; electrophoretic comparisons of a number of proteins; direct sequence data on a few proteins) show that man and the great apes, and in particular, man and chimpanzee, are remarkably similar. These data suggest a time of divergence of perhaps three million years ago, and certainly not the 15–20 million years conventionally accepted. The obvious response has been that protein evolution has been particularly slow since the divergence, perhaps because of the long generation time. Wilson and Sarich retort that there is no evidence in favour of such a view. They argue that absolute dating of common ancestors from the fossil record is unreliable, but that the relative times of branching points are reasonably certain. For example, the common ancestor of man and tree shrew (*Tupaia*) is later in time than the common ancestor of these two and the bear or hyaena. Therefore, if protein evolution in the human lineage has been slow (because of long generation time or for other reasons), then the difference between man and bear should be less than that between tree shrew and bear. In fact, judging by immunological distance, the opposite is the case (although the effect is small). From this and other arguments, they conclude that protein evolution has not slowed down in the human lineage, and that the evidence cannot easily be reconciled with a divergence time greater than five million years.

This conclusion has not recommended itself to palaeontologists. Australopithecines, regarded as well on the way to being men, date back at least three million years. *Ramapithecus*, believed also to be on the human lineage, lived 12 million years ago. Dryopithecines, very similar to modern great apes, date back 20 million years. In the face of this direct evidence, it is hard to accept Wilson and Sarich's conclusion. We ought, however, to remember that *Ramapithecus* is, after all, only a few jaws and teeth, and that one school

of anatomists (noted, it is true, more for panache than perspicacity) still argue that *Australopithecus* is as close to the great apes as it is to man.

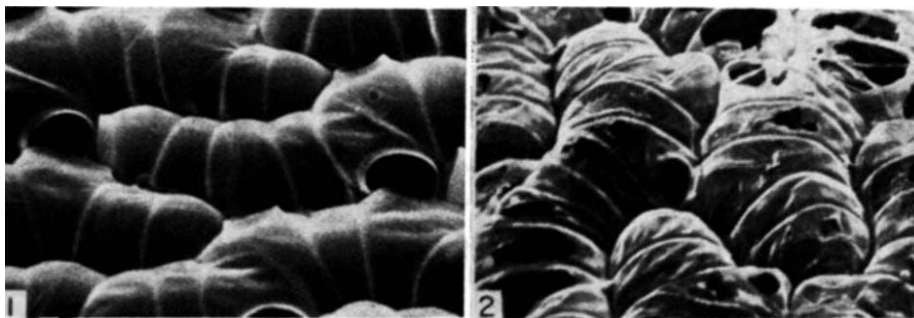
Equally surprising, but less controversial, conclusions have emerged from a comparison of albumin evolution in frogs and mammals. The rate of evolution of albumin has been very similar in the two groups, despite the much greater rate of morphological evolution in mammals (the first fossil frogs are 150 million years old). Wilson, Maxson and Sarich (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2843; 1974) have compared the albumins of 31 pairs of mammal species and 50 pairs of frog species which can produce viable hybrids. The average immunological distances are three and 36 respectively, implying that a tenfold greater degree of differentiation in protein structure is compatible with hybridisation in frogs than in mammals. The authors discuss the possibility that this is because of immunological reactions between mother and foetus in mammals, but give reasons for preferring an alternative explanation. They suggest that hybrid embryos die because of differences in gene regulation between the parental species, and that, relative to evolution of structural genes, gene regulation evolves more rapidly in mammals, associated with their more rapid morphological change. In a later paper (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 3028; 1974) the same authors point out that chromosome evolution (as judged by changes in numbers of chromosomes and of chromosome arms) has proceeded more rapidly in mammals than in frogs and suggest that structural changes in chromosomes are associated with changes in gene regulation.

Decomposition in peatlands

from Peter D. Moore

Most ecosystems in which production is in excess of respiration and decomposition accumulate the surplus energy-rich material as living biomass; peatland ecosystems are peculiar in that their excess is deposited as litter and accumulates as peat. In some peat-forming ecosystems, such as reedswamps, net primary production is high, but in those exhibiting the highest rates of peat accumulation, primary productivity is quite low. In such instances it is reasonable to assume that peat accretion results from a depressed decomposition rate rather than an inflated productivity.

Because mires hold many attractions for biologists concerned with the evaluation of energy and dry matter budgets for ecosystems, and also because peat is



Sphagnum magellanicum branch leaves, convex surfaces showing hyaline cells with supporting bars of secondary wall material and bordered pores. 1, green, living leaf taken from top 0.5 cm of plant; $\times 560$; 2, leaf taken from peat at 10 cm depth, showing partial decomposition of hyaline cells; $\times 460$.

an important natural resource in several countries, it is not surprising that a number of attempts have been made to develop mathematical models of peat-accumulating systems. The efficiency of any ecosystem model is limited by the reliability of the raw data used in its construction and, in the case of peat-forming systems, the most elusive information is that related to decomposition.

It is usually assumed that the loss of organic matter decreases exponentially with time (for example, Gore and Olson, *Aquilo Ser. Botanica*, **6**, 297; 1967). Although such an assumption is reasonable, its confirmation, and a precise knowledge of long-term decay rates for different materials, are extremely difficult to obtain. Clymo (*J. Ecol.*, **53**, 747; 1965) approached the problem by burying nylon mesh bags containing known quantities of *Sphagnum* moss at various depths in the peat. He demonstrated that losses were far higher in the superficial layers of peat than from deeper down, where conditions were permanently anaerobic. The disadvantage of such a method is that in natural circumstances the material entering the anaerobic zone would have already undergone some degradation during its period in the upper peat layers; on this basis one might expect even lower levels of anaerobic decomposition than Clymo's data suggest.

Direct observations on bracken decomposition over a period of years led Frankland (*J. Ecol.*, **54**, 41; 1966) to believe that the course of decomposition was essentially linear, and Reader and Stewart (*Ecology*, **53**, 1024; 1972) have therefore used this assumption in their model of peat accumulation in Canadian muskeg. They use decay rate figures from various plant materials which were exposed in bags for a period of a year. But the apparently linear decay observed by Frankland may have proved to be exponential if observation could have been continued over very much longer periods of time, and the assumption of linear decay is not generally favoured.

Two recent studies of peatland decomposition processes have concen-

trated on microscopic and microbiological examination of peats in order to gain some indication of the nature and rate of the decay process. Dickinson, Wallace and Given (*New Phytol.*, **73**, 107; 1974) have examined peats from the Florida Everglades and used three different methods for determining microbiological activity at different depths. They examined and counted the microorganisms present; they washed, plated and incubated peat samples; finally, they incubated samples from freshly exposed peat surfaces. Direct counts showed large numbers of microorganisms at all depths, but it is possible that the bulk of these were dead. The other two techniques showed a strong concentration of viable organisms within the surface layers of peat, as Clymo's work had suggested for *Sphagnum* peats. Once again, the inference is that the bulk of decomposition occurs in the superficial peat horizons.

An alternative approach has been used by Dickinson and Maggs (*New Phytol.*, **73**, 1249; 1974), who observed the results of decomposition on *Sphagnum* leaves by both light microscopy and scanning electron microscopy (see figure). They devised scores for different degrees of degradation and made estimates of the proportion of *Sphagnum* leaves in each condition at various depths down to 30 cm. Decay was considered to be due to the activity of microflora rather than fauna. Readings were taken at only four levels, making it difficult to discern any precise pattern of degradation with depth; however it would seem that about 20–30% of *Sphagnum* leaves had undergone some degradation at 16 cm depth and about 30–40% at 30 cm depth. Without information about accumulation rates of peat during the time that this 30 cm of peat had built up, it is impossible to infer from these data the precise pattern of decay processes. If, for example, peat accumulation has been fast in the past but is now slowing down it would be possible for material from deep in the peat to be better preserved than that near the surface.

Ultimately the method of Clymo and

Reddaway (*Moor House Occasional Papers*, No. 3; 1971) is likely to prove the most helpful for production ecologists concerned with peatland decay rates. They collected gaseous output from a peatland surface and assessed the flux of carbon dioxide and methane; they also measured organic losses in solution. The gaseous measurements can be considered as the respiratory products of producer, consumer and decomposer organisms from a complete vertical column of the peatland system. Their figures indicate that overall dry matter surplus (available for peat formation) in a Pennine blanket bog varies between $1 \text{ g dm}^{-2} \text{ yr}^{-1}$ on hummocks to $2.7 \text{ g dm}^{-2} \text{ yr}^{-1}$ on *Sphagnum* lawns. In spite of difficulties in measurement and the high variability of data with surface flora and microtopography, this still represents the most promising approach to the measurement of the respiration and potential growth rates of bogs.

Total reconstitution of *E. coli* 50S ribosomes

from a Correspondent

RECONSTITUTION of the *Escherichia coli* 30S ribosome from its constituent RNA and protein has been well established for several years (Traub and Nomura, *Proc. natn. Acad. Sci. U.S.A.*, **59**, 777; 1968), but total reconstitution of the corresponding 50S particle has proved a much tougher nut to crack. A reassembly procedure was described by Maruta, Tsuchiya and Mizuno (*J. molec. Biol.*, **61**, 123; 1971) using proteins liberated by the action of ribonuclease II, but so far no report has appeared of a successful repetition of this result. 50S ribosomes from *Bacillus stearothermophilus* have been reconstituted (Nomura and Erdmann, *Nature*, **228**, 744; 1970) by taking advantage of the higher thermal stability of ribosomes from this organism, but this of course is not so useful since most of the biochemical and genetic information concerning ribosomes has been accumulated from *E. coli*. Hitherto, biochemists have had to content themselves with the 'partial reconstitution' method for *E. coli* 50S ribosomes. This was first reported simultaneously by Staehelin and Meselson (*J. molec. Biol.*, **15**, 245; 1966) and by Hosokawa, Fujimura and Nomura (*Proc. natn. Acad. Sci. U.S.A.*, **55**, 198; 1966) who both showed that protein-deficient 'core' particles could be reassembled to active 50S ribosomes. Now, however, the total reconstitution of *E. coli* 50S particles from protein and RNA has been achieved, by a two-step incubation procedure, and is reported by Nierhaus and

Dohme in *Proc. natn. Acad. Sci. U.S.A.* (**71**, 4713; 1974).

Nierhaus and Dohme began by finding out how many proteins could be removed from the 50S particle before the resulting core particle became unable to regain its activity in the partial reconstitution procedure. Activity was measured both by the poly(U) assay, and by the 'fragment assay' for polypeptidyl transferase. They found that whereas 4 M LiCl cores could still regain activity, 5 M cores (containing only five proteins) had largely lost this ability, and 6 M cores (with only two proteins) could not be reconstituted at all. The obvious next step—a stepwise reassembly of 23S RNA to a 4 M core particle, and thence to a 50S ribosome—was not successful as judged by the fragment assay, although some activity in the poly(U) assay was recovered. Nierhaus and Dohme rightly interpreted this failure as an indication that the ionic conditions of the partial reconstitution procedure were not ideal for the early stages of total reconstitution.

They therefore mixed 23S RNA, 5S RNA and total protein, and investigated the effect of various incubations prior to the normal partial reconstitution. By optimising the conditions they arrived at a two-step procedure in which the isolated ribosomal components were first incubated together for 20 minutes at 40°C in 4 mM magnesium, 400 mM ammonium at pH 7.2, followed by the partial reconstitution incubation of 90 minutes at 50°C in 20 mM magnesium, 400 mM ammonium. This gave particles with a remarkably high activity, that is to say 80–110% in the poly(U) assay, and 40–60% in the fragment assay, as compared with native 50S sub-particles. Notably, no requirement for 30S particles or polyamines in the reconstitution mixture was observed. Phenol-extracted RNA and acetic acid-extracted proteins were used in the procedure, in order to ensure that the RNA fraction was entirely free from protein and the protein fraction entirely free from 5S RNA. This can therefore be regarded as a genuine total reconstitution, and should prove as advantageous to the understanding of the 50S particles as the corresponding reconstitution has already proved to the 30S. Detailed 'assembly mapping' of the 50S particle should now follow without too much difficulty.

Pituitary neuropeptides and behavioural processes

from A. Dickinson

THE idea that pituitary neuropeptides can influence behaviour by a direct action on the central nervous system is

a recent one. These effects of pituitary hormones seem to be distinct from their well known actions on target tissues such as kidney and adrenal cortex; they resemble rather the actions on the CNS of steroid hormones secreted by endocrine target tissues.

The relation of pituitary neuropeptides to learning processes was one of the topics discussed at the fourth winter school of the European Training Programme in Brain and Behaviour research (January 4–11). Some of the strongest evidence for a direct action on the CNS comes from the work of the Utrecht group on the effects of adrenocorticotrophic hormone (ACTH) and related peptides on avoidance behaviour. In their experiments an animal is required either to make some response (active avoidance) or to refrain from responding (passive avoidance) in order to avoid an electric shock. They showed previously that injections of ACTH restored the deficit in active avoidance induced by hypophysectomy and prolonged active avoidance in intact animals during extinction. ACTH also improves passive avoidance acquisition. The important finding is that these behavioural effects seem to be independent of the adrenocorticotrophic activity of ACTH since the injection of ACTH 4–10, a fragment of the ACTH peptide chain which has no adrenocortical stimulating activity, produces comparable changes in avoidance behaviour.

Dr B. Bohus's report on this work made it clear that the facilitatory influence of ACTH was only seen while the hormone was present in the body. It was therefore suggested that the primary central effect of ACTH is to increase the general arousal or motivational state of the animal. Such a general effect is in line with biochemical studies reported by Dr W. H. Gispen (Institute of Molecular Biology, Utrecht) showing that the behaviourally effective fragments of ACTH seem to increase the incorporation of labelled leucine into almost all brain stem proteins of the rat.

Dr J. A. Gray of the University of Oxford reported a complementary series of experiments using learned appetitive responses rather than avoidance responses. After training approach responses by positively reinforcing them with rewards such as food and water he extinguished the responses by withdrawing the reward and found that ACTH 4–10 prolonged such extinction. Though at first sight his results seem to be comparable to those of the Utrecht group, it is not clear that the interpretation given above for the Utrecht results will explain those of Dr Gray. Withdrawal of a reward engenders emotional arousal such as frustration; such aversive arousal actually

helps to produce extinction and an ACTH-induced increment in arousal might therefore be expected to shorten extinction of a positively reinforced response while prolonging the extinction of avoidance behaviour.

Dr Gray has provided further evidence that ACTH attenuates the emotional responsiveness of an animal as measured by positively reinforced responses. Tranquillisers such as sodium amylobarbitone seem to produce the same behavioural effects during the extinction of these responses as exogenous ACTH. Furthermore, Dr Gray has shown that the ACTH 4-10 fragment increases the threshold for driving a specific electrical rhythm of the hippocampus (the theta rhythm) from the septum in the same manner as sodium amylobarbitone. Previous Oxford work has implicated this system in the mediation of the behavioural effects of nonreward.

A second pituitary hormone with interesting behavioural effects is vasopressin. The Utrecht group has shown that this neuropeptide and its analogues without antidiuretic or pressor action have effects similar to those of ACTH on avoidance behaviour. They differ from ACTH in having an influence which lasts longer than their presence in the body. Dr Bohus suggested that vasopressin influences memory processes by affecting the consolidation or retrieval of learned responses. Using a passive avoidance task in which rats learned to suppress a response in one trial, he reported that animals with a lack of vasopressin through hereditary hypothalamic diabetes insipidus showed good retention if tested immediately after training but not if testing was delayed for three or twenty-four hours. If registration and storage-retrieval processes really can be distinguished using this neuropeptide, it may provide a powerful tool in the analysis of learning and memory.

In the study of the pituitary neuropeptides, psychological theory meets fruitfully with the other neurosciences. The effects of exogenous ACTH on the performance of learned tasks, effects which do not fit easily into the current behavioural analyses of these tasks, provide an important guide for revising both behavioural theories and concepts about the sites and modes of action of neuropeptides in the CNS.

Chilling statistics on Cyprus

from Peter J. Smith

THE sheeted diabase unit of the Troodos complex in Cyprus is a complicated geological feature whose stratigraphically central section is formed completely of dykes intruding other

dykes. The unit as a whole has an exposure in the east-west direction of more than 70 km, although the individual dykes, which generally strike north-south, range in width from a few metres to only a few centimetres. The periods between dyke injections were apparently sufficient to allow the previous dykes to cool, so that each new dyke was chilled against those into which it was intruded and thus has finer-grained or cryptocrystalline margins. But as many of the dykes are intruded by later ones, they may have been split several times, forming apparently marginless dykes which are in reality merely the central parts of once complete intrusions. And if that is not enough to cause confusion, some dykes intruded up the margins of previous dykes, so that the later dykes were chilled against the chilled margins of the earlier ones.

What is the origin of this remarkable structure? The answer to that question would be interesting enough if the diabase unit were only of purely local significance. But the Troodos complex is, of course, an ophiolite suite comprising ultramafics, gabbro, sheeted dykes, pillow lavas and deep sea sediments—a feature widely believed to represent ancient ocean floor. If the origin of the diabase unit could be determined, the result would give by implication the origin of the whole interrelated complex; and geologists would be sure at last whether or not they could claim access to ocean floor without the need for deep ocean diving.

The answer most geologists would welcome would be proof that the diabase dykes were injected at, or close to, an oceanic spreading axis. But as Kidd and Cann (*Earth planet. Sci. Lett.*, **24**, 151; 1974) point out, a structure composed entirely of dykes could be produced in two other ways—by random injection throughout the whole structure (that is, with a zone of intrusion much wider than that implied by seafloor spreading) or by the coalescence of dyke swarms from individual volcanoes.

Fortunately, however, the three models do not predict the same detailed dyke swarm structure. Consider the case of ideal seafloor spreading, in which the width of the zone of intrusion is infinitely small and in which each new dyke intrudes up the centre of the preceding one. On either side of the intrusion zone the oceanic crust will comprise entirely half-dykes, each having a chilled margin on the side away from the intrusion zone. In other words, on each side of the intrusion zone the magnetic vectors within the chilled margins will all lie in the same direction (they will be all 'one way') and the degree of 'one way chilling' will be 100%.



A hundred years ago

THE *Kölnische Zeitung* of Feb. 7 contains an abstract of a paper read by M. G. Wex, at the Geographical Society of Vienna, on the decrease of water in rivers and sources. The author states that the results of his observations tend to show the constant decrease of the rivers of Germany and the increase of seas. It appears from them that the levels of the German rivers are now much lower than they were fifty years ago; viz., the Elbe 17 in., the Rhine 24.8 in., the Oder 17 in., the Vistula 26 in., the Danube 55 in. As a reason for this decrease, the author gives the progressing devastation of forests, which causes a decrease in the atmospheric moisture they attract and convey to the soil and thence to sources. from *Nature*, **11**, 314; February 18, 1875

In any real spreading system, complete 'one-way chilling' will never be achieved because the intrusion zone will have a finite width and the splitting of previous dykes will not be as regular as in the ideal case. Nevertheless, as long as the intrusion zone is not too wide, some of the dykes will have been intruded close enough to the centre of injection for them to have been split by subsequent injections; and in such a case the degree of one way chilling, though far from 100%, should be significantly greater than zero. It is this statistical point which should enable the seafloor spreading model to be differentiated from the other two. Where intrusion takes place throughout the whole dyke region or where it is derived from several discrete volcanic centres, there is no single effective centre of injection and thus presumably no bias in the chilled margin directions.

Kidd and Cann have now applied these ideas by taking a number of well exposed and widely scattered sections within the Troodos dyke unit and measuring the directions of chilling of an odd number of margins within each section. Because the number of chilled margins within each section is always odd, there is bound to be an excess of margins in one direction or the other (east or west in this case because the margins trend north-south). But whereas the seafloor spreading model would predict a significantly greater number of sections with an excess in one direction rather than the other (the one direction being that away from the spreading centre), the two other models would predict no such imbalance.

In practice, the statistical bias is confirmed. Out of 23 section transects covering 41 chilled margins each, 19 transects (TRE) showed a preponderance of margins chilled to the east over margins chilled to the west and 4 transects (TRW) showed a preponderance the opposite way. The chance of obtaining such a bias from a system which actually contains equal numbers of TREs and TRWs is less than 1 in 200. This suggests that the statistics reflect a real physical effect, namely, that the Troodos dyke unit was produced as part of the seafloor spreading process. This conclusion is further supported by the number of chilled margins actually in excess within each transect. For transects with margins predominantly to the east the average number of margins in excess within each transect is 2–3, whereas for transects with margins predominantly to the west the corresponding figure is only 1.

Finally, since the predominant chilled margin direction is the direction away from the spreading centre, it follows from the data above that the spreading axis should have lain relatively to the west of the Troodos complex. It is necessary to say 'relatively' here because Moores and Vine (*Phil. Trans. R. Soc.*, **A268**, 443; 1971) concluded from palaeomagnetic evidence that the complex has rotated through 90° in an anti-clockwise direction since it was formed, which implies that the dykes within the diabase unit were originally intruded in an east-west direction and that any spreading axis would have lain to the north. But although the orientation of the supposed spreading system is important, it is far more important for the time being to show that spreading took place at all. The simple, but elegant, piece of work carried out by Kidd and Cann provides further evidence that it did.

Copernicus and X-ray astronomy

from A. C. Fabian

THE Copernicus satellite launched in 1972 carries an 80 cm ultraviolet telescope-spectrometer from the Princeton University Observatory measuring interstellar absorption lines superimposed on stellar spectra. The small companion X-ray package from the Mullard Space Science Laboratory at University College, London, contains three grazing-incidence telescopes and a collimated proportional counter of about 15 cm² aperture. Both sets of instruments are pointed to the same location in the sky to fractional arc second accuracy by the inertial guidance system. Thus the ultraviolet telescope sometimes observes X-ray sources, and the X-ray systems spend

some of the time viewing hot stars.

In spite of this apparent clash of interest, much of the work with the X-ray package has depended upon the Princeton telescope. Fifty per cent of the light incident on the Princeton spectrometer is used in a fine error sensor to provide the primary pointing control for the satellite and a stellar reference system for the X-ray experiment. The resultant accurate pointing has allowed maps of supernova remnants and clusters of galaxies to be made. Scans of a number of X-ray sources in our Galaxy and the Large Magellanic Clouds have enabled their positions to be pinpointed within a few arc minutes. Identification with optical, radio or infrared counterparts is proceeding.

The failure of two of the X-ray telescopes in July 1973, owing to a jammed shutter blocking the light paths, has caused mapping operations to cease. Nevertheless, the collimated proportional counter which operates over a 2.5–10 keV range has not been idle. It is well known that the binary X-ray sources are highly time variable. The stable platform provided by Copernicus has allowed careful studies to be made of some of these X-ray binaries. The data so obtained are superior to those from scanning instruments such as *Uhuru* on time scales exceeding several minutes, owing to this accurate pointing. The larger area X-ray detectors on the recently launched Ariel-5 that view along the spin axis also enjoy some pointing stability, but drift of the spin axis will introduce uncertainties.

Source identifications can still be made through correlated X-ray and optical or infrared observation. Lunar occultations also provide a powerful technique, and have been successfully applied from Copernicus to a galactic centre source, GX5-1 and the Crab nebula. The motion of the satellite allows several passages of the Moon across these sources to be observed.

Many more X-ray observations have been proposed for 1975. At least one complete 'on'-state of the 35-d cycle of Hercules X-1 will be followed. This enigmatic binary system has been intensively studied optically over the past two years but the mechanism behind its 35-d cycle is still unclear; complete and accurate X-ray data are lacking. Several other binaries and suspected binaries will be monitored for extended periods. The tight schedules of the other X-ray detectors in orbit do not permit extended study of particular objects because of the need to conserve stabiliser gas in the case of UK-5 and the viewing constraints for the ANS. It is hoped that the X-ray observing programme of Copernicus will continue, for there is no lack of sources wanting investigation.

Nuclear spectroscopy

from P. E. Hodgson

STUDIES of nucleon transfer reactions have long been established as one of the most powerful nuclear spectroscopic techniques. The angular distribution is characteristic of the angular momentum transfer L and the magnitude of the cross section is a measure of its single-particle strength. If the target nucleus has spin zero and the transferred particle is initially in a state of zero orbital angular momentum the spin of the final state is simply given by the vectorial sum of the relative orbital and spin angular momenta in the transfer process. For a (d,p) reaction, for example, $\mathbf{J} = \mathbf{L} + \frac{1}{2}$ giving $J = L \pm \frac{1}{2}$. Thus a $L=2$ transfer can go to a $J=3/2$ or to a $J=5/2$ state. The ambiguity in sign can often be resolved from the known systematic behaviour of single-particle states but in other cases it remains a difficulty. Measurement of the polarisation of the outgoing proton together with distorted wave calculations or a simple comparison with the proton polarisations in reactions leaving the residual nucleus in states of known spin resolves the ambiguity, but is time consuming.

It has however proved possible in some cases to resolve the ambiguity by examination of certain features of the differential cross sections and it is found that they are characteristic of $J=L+\frac{1}{2}$ or $J=L-\frac{1}{2}$ final states. Such features are sometimes difficult to reproduce by distorted wave calculations but nevertheless can be used as empirical guides provided that they can be calibrated by studies of transitions to states of known spin.

These J -dependent effects, as they are called, are sufficiently distinctive to be used with confidence only for particular reactions in restricted energy ranges, so it is always valuable to find new circumstances or new reactions that display them prominently. Two recent papers have provided evidence of this type.

In the first paper, Kong-a-Siou and Chien of Michigan State University (*Phys. Lett.*, **52B**, 175; 1974) measured the angular distributions of several $L=3$ transitions in the (p,d) reaction on ⁶¹Ni and ⁶²Ni at 40 MeV and found a very stable J dependence over a range of intensities and excitation energies. Some of their results are shown in Fig. 1, and it is clear that the angular distributions fall sharply into two classes, one characteristic of $J=L-\frac{1}{2}=5/2$ and the other of $J=L+\frac{1}{2}=7/2$, and that in each case the data fall quite definitely on one or the other. The data for ⁶²Ni(p,d)⁶¹Ni thus allow the spins of the states of ⁶¹Ni to be determined.

The results for ⁶¹Ni(p,d) ⁶⁰Ni to states

Palaeoscience and the modern world

from J. A. Taylor

WHAT relevance have the palaeosciences to the interpretation and solution of the environmental problems of the modern world? Judging by the niggardly support given to some of the palaeosciences (notably palaeobotany, palaeopedology and palaeoclimatology) by the Natural Environment Research Council, the answer would be "precious little". Yet the improved techniques of the palaeosciences are finding uses in present-day ecological studies, thus bridging the gap between the study of ancient and modern environments. At a symposium of the Biogeography Study Group held in Oxford on January 6 methods were described for absolute dating, and for dating very recent objects. Palaeomagnetic dating and lead and caesium analysis are now becoming available to supplement pollen records and radiocarbon assays, already corrected for errors detected by dendrochronological research. Again, in the field, magnetic susceptibility measurements are proving an invaluable aid in selecting representative single cores from extensive peat bogs, lake basins or peaty moorlands.

F. Oldfield (University of Lancaster) reported the preliminary but successful application of these new techniques in environments as contrasted as Sweden and New Guinea. His advocacy of the lacustrine sediments as a superior source of continuous and complete palaeochronologies is, however, open to some criticism. Such sediments may incorporate successively younger layers, duplicated or polycyclic layers, unconformities due to erosion, and homogenisation, due to alluvial and colluvial mixing, as B. Seddon (University of Reading) reported for his detailed pollen record for sediments at Llangorse lake in Powys, Wales. Clearly, differentiation between lacustrine and terrestrial sources and between riverborne and airborne pollen is necessary. It is thus desirable to examine modern pollen cycling within the adopted lake catchment and to analyse soil pollen records and pollen evidence from peats and muds.

Several papers described local palynological studies which have regional significance in Britain. It is beginning to be possible to reconstruct vegetation cover for different culture periods. Some changes, such as elm decline, are broadly synchronous (though substantial variations in timing occur at certain Welsh sites); others, such as man-made clearances between Mesolithic times and the Iron Age are more diachronous. It is becoming clear that late Mesolithic man was considerably

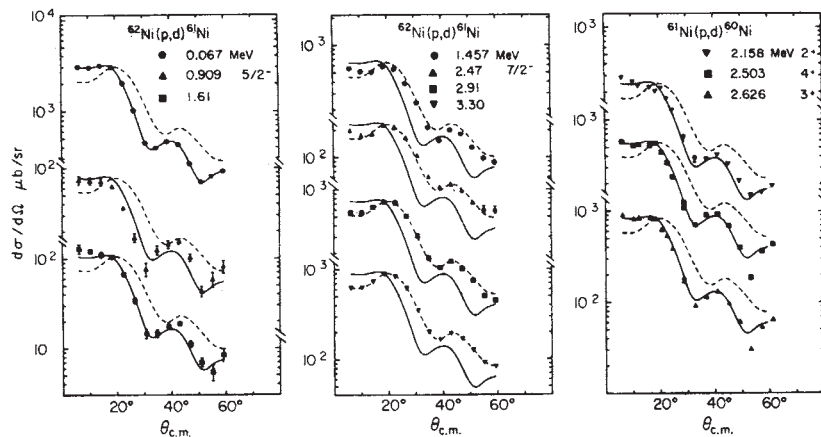


Fig. 1 Differential cross sections for the $^{61}\text{Ni}(p, d)^{60}\text{Ni}$ and $^{62}\text{Ni}(p, d)^{61}\text{Ni}$ reactions. The solid and dashed lines are smooth curves through the experimental data points of the known $f_{5/2}$ and $f_{7/2}$ transfers to the states at 0.067 and 3.30 MeV in ^{60}Ni . All the cross sections follow closely one curve or the other.

of known spins 2^+ , 4^+ and 3^+ respectively show angular distributions characteristic in each case of quite pure $J=5/2$ transfer. For the 4^+ state, only $L=3$ transfer is allowed by the conservation of angular momentum and of parity, but for the 2^+ and 3^+ states both $L=1$ and $L=3$ transfers can complete. The data show that only $L=3$ is present and this can indeed be understood by shell-model calculations.

The second paper, Kemper *et al.* of Florida State University (*Phys. Lett.*, **52B**, 179; 1974) report a study of the relatively unfamiliar (^7Li , ^6He) reaction that shows marked J -dependent effects for reactions to d and f states. For this reaction the transferred proton comes from the $p_{3/2}$ orbit in ^7Li so that the vector equation for the angular momenta is $3/2 + l = J$, where l is the orbital angular momentum of the transferred proton and J the spin of the final state. If for example $J=3/2$, then l can be 0, 1, 2 or 3. If the final state has orbital angular momentum L , then J can be $L \pm 1/2$ and the possible values of l are $l=L$ or $L \pm 1$. The relative proportions of these that contribute are given by the Racah coefficient

$W(L, J, 1, 3/2; 1/2)$ and it turns out that the components $l=L$ and $l=L-1$ are much more important for the states with $J=L+1/2$ than for those with $J=L-1/2$.

This is shown clearly in Fig. 2 by the angular distribution for the $^{24}\text{Mg}(^7\text{Li}, ^6\text{He})^{23}\text{Al}$ reactions at 34 MeV to $d_{3/2}$ and $d_{5/2}$ states of ^{23}Al . The curves show separately the distorted wave calculations of the contributions to the two cross sections, and it is found that the reaction to the $J=3/2$ state ($J=L-1/2$) is nearly all $l=3$ with very small contributions from $l=1$ and 2, whereas for the reaction to the $J=5/2$ state the $l=1$ and 2 components are relatively much more important. Since the contributions of different l have markedly different angular distributions this gives a difference between the cross sections for the reactions to the $J=3/2$ and $J=5/2$ states, and this can be used to distinguish between them.

Reactions like this are only just beginning to be studied in detail, and it is likely that other types of J dependence will be found and that they will prove powerful tools in nuclear spectroscopy.

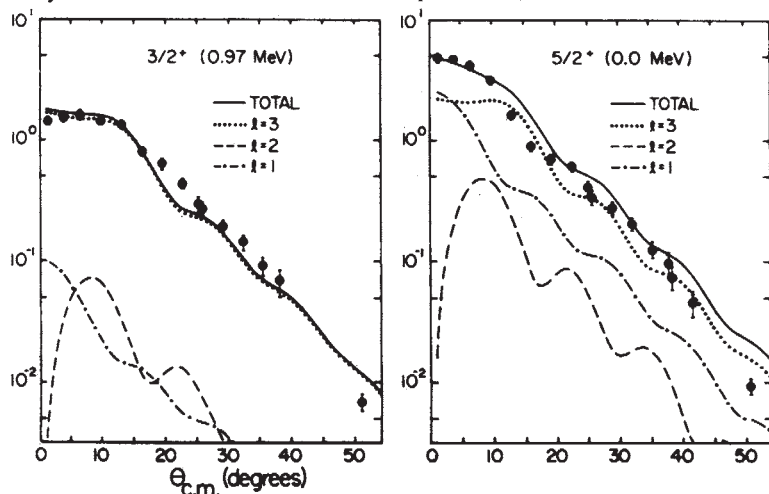


Fig. 2 Differential cross sections for the $^{24}\text{Mg}(^7\text{Li}, ^6\text{He})^{23}\text{Al}$ reaction to $d_{3/2}$ and $d_{5/2}$ states in ^{23}Al . The curves are distorted wave calculations and show the difference between the relative contributions from $l=1$, 2 and 3 transfer in the two cases.

more advanced than archaeologists have previously believed. He partially domesticated the red deer and initiated sizeable forest clearances.

In comparing old, middle-age and young soils on Exmoor K. Crabtree (University of Bristol) and E. Maltby (University of Exeter) estimated the developmental stage reached by a soil profile by measuring the rate of accumulation of organic matter over time. By this technique again a process can be calibrated from its inception to the present day; eventually absolute soil chronologies should be available which will allow refined measurement of the ecological stability and trend of individual soil environments as related to management and planning.

The problems which recur when attempts are made to interpret archaeological evidence in environmental terms were well exposed by D. A. Davidson (University College, Lampeter) and R. L. Jones (Lanchester Polytechnic) who examined the location and distribution of Neolithic chambered cairns in the Orkneys. A model for the island of Rousay evaluated seven environmental parameters of cairns: distance from coast, nature of coast, slope, soil drainage, rockiness, altitude and visibility from the mainland of Orkney. The sites generated were compared with the actual distribution of cairns and the best approximations were found to occur for (1) nearness to a beach coastline and (2) steepness of slope. W. Kirk (Queen's University, Belfast) criticised any rationalisation of the location of cairns since they were burial places and not settlement sites. But as Frances Lynch (University College of North Wales, Bangor) has argued (in *The Effect of Man on the Landscape: Highland Zone*; Council for British Archaeology Research Report II, in the press), prehistoric man may have been discriminating in the selection of both vantage points and viewpoints for burial as well as settlement. A subtle blend of the defensive and the exhibitionist, and of the imitation of natural features, may sometimes be inferred.

Lunar eclipses and Danjon's law

from David W. Hughes

LUNAR eclipses occur when the Earth's shadow is cast over the full Moon. They have an average duration of 226 min, totality (full shadow) lasting about 103 min. Sixteen lunar eclipses occurred between 1965 and 1975; the exact time of occurrence depends on the relationship between the draconic (lunar nodal) month, the synodic (identical phase) month, the precession of the nodes of

the orbit and the Earth-Moon-Sun geometry. In totality the Moon presents a fascinating spectacle—the contours of the seas and some of the craters are discernible, its hue is distinctly red and its brightness and colour vary from limb to centre creating the impression of a sphere as opposed to the flat disk of the normal full Moon.

The reddish glow is an interesting phenomenon. Rays of sunlight just grazing the edge of the Earth's globe must pass right through the thickest part of the atmosphere where they undergo refraction and are bent into the cone of shadow and fall upon the eclipsed Moon. The light also undergoes absorption due to Rayleigh scattering; the short wavelengths are attenuated much more than the long, leaving the remaining light red like sunsets on Earth. The spectrum of the light reflected from the eclipsed Moon and its variation across the disk can be explained by the absorption of light in the terrestrial ozone layer and can be used to give a concentration-height profile of ozone in the Earth's atmosphere.

In 1920 Danjon, a French astronomer, noticed a relationship between the phase of solar activity and the brightness of a lunar eclipse (*C. r. Acad. Sci. Paris*, **171**, 1127; 1920). This relationship is now known as Danjon's law and can be stated as follows: in the two years immediately after a solar activity minimum the shadow of the Earth during a lunar eclipse is very dark and has little colour. As the solar activity moves away from minimum the eclipsed Moon becomes brighter and redder until, during the seventh and eighth year after solar minimum the eclipsed Moon is at its brightest and is red, copper coloured or orange. The brightness curve then falls away very sharply to its minimum value. The maximum phase of the solar cycle passes unnoticed whereas the minimum phase is indicated by a sudden and considerable diminution in brightness and colour, this change forming a discontinuity.

To obtain this law Danjon had analysed eclipse records dating back to the time of Tycho Brahe. He subsequently used the law to estimate the times of solar minimum during the 17th, 18th, and 19th centuries, finding the general relationship that minima occurred in the years $1584.8 + 10.87n$ where n is an integer (*C. r. Acad. Sci. Paris*, **171**, 1207; 1920). Notice that the length of the solar cycle, 10.87 years as derived from lunar eclipses, is less than the currently used value of 11.2 years derived from sun spots.

Link, of the Institut d'Astrophysique, Paris, has reconsidered the underlying causes of the variations of lunar eclipse brightness described by

Danjon's Law in a recent edition of *The Moon* (**11**, 137; 1974). He finds that the phenomenon behind the law is not the absorption of the light in the terrestrial atmosphere, as originally assumed by Danjon, but some light source other than the normal refracted solar illumination. The problem is to find this source. Vassy (*J. Sci. Mét.* **8**, 1; 1958) hypothesised that the cause is a scattering layer in the Earth's upper atmosphere produced by aerosols. The spatial density of these aerosols could be affected by the corpuscular radiation flux from the Sun and the variation in density caused by the sudden change in the heliographic latitudes of the source of this radiation after solar minimum. Link examines the possible implication of this hypothesis on the twilight and daytime sky as observed from Earth. Looking at two extremes of shadow density he finds the eclipse of January 18, 1954 to be a thousand times less luminous than that of September 26, 1950. To explain the bright eclipse by aerosol scattering requires an aerosol density such that the twilight brightness would be a hundred times more than observed. The luminance of the daylight sky too is far from that needed to explain Danjon's Law.

Another source of additional light is the luminescence of lunar surface materials. This could be caused by corpuscular radiation striking the Moon during the eclipse or it could be an afterglow from the period preceding the eclipse. But recent laboratory examinations of lunar samples showed that the luminescence is much too low to explain the eclipse observations.

Two other facts must be borne in mind: first Fisher (*Smithson. Misc. Coll.*, **76**, No 9; 1924) found winter eclipses to be brighter than those occurring at other seasons and second, exceptions to Danjon's Law have occurred when very dark eclipses were seen after the eruption of the volcanoes Krakatoa (1883) and Katmai, Alaska (1913).

But we must return to Link's final conclusions; that a scattering layer in the upper atmosphere, or luminescence of the lunar surface—both caused by solar corpuscular radiation—describe qualitatively the general features of Danjon's law but are quantitatively completely unsatisfactory. So the cause of the relationship remains unknown.

Erratum

In the report "G-wizardry at Dallas" by John Faulkner (**253**, 231; 1975) the scalar coupling constant was printed in the penultimate paragraph as ≥ 23 instead of $\lesssim 23$. Also Colgate's institution is New Mexico Institute of Mining and Technology, not University of New Mexico as printed.

THE central theme of the symposium was structure at the molecular level. Many fibrous proteins have a highly regular structure; for example, the collagen of tendon shows nature adopting at the molecular level the principle of successive twisting that man has developed in the rope. Man has not yet developed a molecular rope, but different methods of drawing polyethylene lead to fibres with a wide variety of mechanical properties, some with the stiffness of glass and the tensile strength of steel (Ward, Leeds). The mechanical properties may be related to the structural regularity of the molecular folds in the polymer. There is a striking similarity between the folded chains of polyethylene (Sadler, Bristol) and those of the protein in the egg stalk of the lacewing fly, *chrysopa*, but many natural polymers share with the synthetics an intermingling of regular and less regular structural regions. In the globular enzymes, the same types of chain conformation are found as in fibrous proteins, but each stretch is limited in extent so that the chain can fold to form a compact shape (North, Leeds). The recurrence of certain well defined folds in different enzymes probably indicates evolution from a common ancestral protein, though there remains the possibility that some conformations may recur just because they are energetically favourable, as do the folds in polyethylene.

The alternation of regularity with irregularity is seen too in polysaccharides, the formation of gels of which depends upon molecular chains being associated in pairs for limited distances (Rees, Unilever); interruption of the regular alternating copolymer by a sugar unit with a different conformation makes a kink in the chain, causing the molecular partnership to break and the two chains to diverge before joining up with different partners. The variety of sugar units utilised in polysaccharides permits a great range of conformations, some of which are very susceptible to changes in solvent environment (Atkins, Bristol); thus conformational changes similar to those induced in heparan sulphate by calcium may be a vital factor in cell adhesion.

In the chromatin of the cell nucleus, the nucleic acid is intimately associated with histones, basic proteins of five types. It is now beginning to appear that the histone molecules are individually globular and associate specifically to form a bead-necklace around which the nucleic acid is wound (Thomas, Cambridge).

A second theme of the conference was chemical modification of polymers. Technologists may attempt to modify a polymer in order to improve its properties, such as the drape and

Natural and synthetic polymers

With the praiseworthy intent of allowing enzymologists and polymer scientists to learn from each other, the SRC Enzyme Chemistry and Technology Committee organised a meeting on January 2 and 3 at Cambridge. In three sessions the meeting dealt with synthetic polymers, structural natural polymers and enzymes. The reports of the meeting which follow describe it from the point of view of, respectively, an enzymologist and a polymer scientist.

crease-resistance of cotton fabrics (Roberts, Shirley Institute). Protein workers often use chemical modification to a different end, in probing structure and function. Very high degrees of specificity may be achieved (Dixon, Cambridge), though care must be exercised in drawing conclusions from the effects of such modifications—the activity of an enzyme may be perturbed by changes remote from its active site. Such methods have been applied elegantly in distinguishing the part played by individual subunits in a multimeric enzyme and in studying the interactions between components of a multi-enzyme complex and between the subunits of a virus (Perham, Cambridge).

The Cambridge meeting established interesting overlaps between the work on natural and synthetic fibres, for example in the molecular basis of mechanical properties and in the thermodynamic and kinetic constraints on the folding pathways. While the programme had a bias towards the natural fibres, showing clearly the great subtlety, elegance and efficiency with which nature has evolved a wide variety of functions from a limited repertoire of structural elements, it is clear that man has already achieved enormous progress in a very short time in making an almost comparable variety of synthetic materials.

Experiments with model compounds (Kirby, Cambridge) show how chemical reaction rates depend critically upon the reacting groups being constrained into an appropriate geometry; such studies are vital to the eagerly awaited development of catalysts with the efficiency and specificity of enzymes—which will doubtless owe much to comparative studies of natural and synthetic polymers.

A. C. T. NORTH

SCIENTISTS have great trouble even in communicating the meaning of their work to each other. This was probably the main conclusion to be drawn from the symposium which, because of the lack of dialogue or (apparently) of flashes of inspiration, must be judged a failure. The reasons may be that the speakers did not address themselves sufficiently to areas of potential overlap, that the discussion was too limited or that the two subjects are irrelevant to each other.

Polymer science groups in the USA are increasingly turning their interest to biological macromolecules, spurred on by the increase in the fortunes of the NIH compared to those of the Defense Department a few years ago. This has resulted in work on solid state and solution properties of polysaccharides and polypeptides, one obvious avenue, and in the study of collagen ultrastructure and its relationship to mechanical properties presented by R. G. C. Arridge and L. Gathercole of Bristol and carried out jointly with Case Western Reserve University.

One thing that did emerge was the relative lack of understanding of physical aspects of enzyme structure such as the denaturation and renaturation processes, the nature of subunit interactions, and of questions such as "How large must a protein be to have a hydrophobic core?" posed by T. L. Blundell during the discussion. This was reinforced by the parallel between J. O. Thomas's review of work on chromatin structure at Cambridge and D. M. Sadler's of polymer crystallisation and morphology. Both topics are almost baroque in their complexity, the methods used are completely different, yet the problems are very much related. It is to be hoped that researchers starting from the synthetic polymer direction can contribute to these subjects, though the immediate problem for a person used to working with kilograms of sample is to learn techniques for handling natural polymers, and here cooperation between the groups is essential.

P. D. CALVERT

articles

The two geological time scales

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Several international bodies are currently preoccupied with the task of establishing, defining and standardising time scales for geology. Geological ages may be expressed on either of two alternative scales often distinguished somewhat misleadingly as 'relative' and 'absolute' ages.

THE chronostratigraphic scale (with its hierarchy of eon, era, period, epoch, age, and chron) has developed slowly over more than 150 years. Its divisions were initially unconformities, conceived of as natural chapters in Earth history, but these were later superseded by divisions defined palaeontologically. The scale is currently being redefined on a global basis, with boundaries standardised at reference points in type sections, in order to produce a Standard Stratigraphic Scale¹ or Standard Global Chronostratigraphic (Geochronologic) Scale². The principle has been illustrated by the establishment of one point on the scale—the Silurian–Devonian boundary³.

The geochronometric scale⁴, unlike the chronostratigraphic scale is based on a unit of duration, originally defined as the geochrone⁵, but since the development of radiometric methods the terrestrial year has been the only unit in general use. The geochronometric scale is periodic, that is, simply the numerical multiplication in steps of 1,000 of the agreed unit (10^0 yr = 1a; 10^3 yr = 1ka; 10^6 yr = 1Ma; 10^9 yr = 1Ga). This scale is already in use whenever an age is expressed in years. The geological year is not yet standardised as it is necessary to choose between the Ephemeris 1900 year of the International Union of Astronomers 1957, and the year based on the caesium second of the International Conference of Weights and Measures 1957; but that is of theoretical interest only¹. Of practical interest, however, is the often expressed wish to divide the whole scale into longer spans of time with internationally agreed names.

The terminology used for the two scales is in some doubt: for many years the term 'geochronology' has been used mainly within the context of the geochronometric scale (for example, IUGS Subcommittee of Geochronology), whereas the IUGS Subcommittee of Stratigraphic Classification (following the American Stratigraphic Code) has used the same term when referring to time spans of the chronostratigraphic scale². To avoid confusion the term 'geochronometric' can be used unambiguously for a scale based on years as the term 'geochronology' might become used generally with both scales.

Both are framework scales⁶; they both express time but in quite different ways. They are both artificial; they cannot be discovered but only decided by convention (for example, 'golden spikes' for the chronostratigraphic scale, and a standard second for the geochronometric scale). The framework allows the expression of the ages of phenomena in a common language and the phenomena (biological evolution, magnetic reversals, climatic fluctuations, tectonic events, nuclear decay, and so on) are necessary for correlation and the 'determination' of age. It is an historical consequence and not a scientific necessity that previously has led biostratigraphy to be concerned largely

with the development of the chronostratigraphic scale whereas with isotope geology the geochronometric scale has been more important. Whereas all methods may be useful in connection with either scale, each of which has its distinctive advantages, metazoan fossils and precise biostratigraphy are limited to the last seventh of Earth history and that has resulted in the almost exclusive use of the chronostratigraphic scale by Phanerozoic geologists whereas Precambrian geologists use mainly the geochronometric scale.

I suggest that this is neither a necessary nor a useful restriction and that more active steps should be taken to extend the use and range of each scale independently. Until that is done we are liable to use a hybrid scale^{7,8}—Precambrian geochronometric and Phanerozoic chronostratigraphic—the latest Precambrian division being a hybrid with its beginning defined in years (whatever that means in terms of geological history) and its end defined in an initial Cambrian stratotype (whatever its age in years). This hybrid needs rooting out along with the many other "weeds in the Precambrian rock garden"⁹. It inhibits the proper development and use of each scale if each, instead of coexisting with the other, serves only one part of a single scale⁸. In parallel they supplement each other and invite mutual calibration¹⁰.

Extension of the chronostratigraphic scale

For some time the methods so successful in Phanerozoic stratigraphy have been applied to Precambrian rocks; and

Table 1 Chronostratigraphic scale

Eon	Era	Period	Lower divisions	Approximate position on geochronometric scale (Ga)
	Cenozoic			—0.065±
Phanerozoic	Mesozoic			—0.225±
	Palaeozoic			
		Cambrian		
		↑		? —0.57±
		Ediacaran*		
	Vendian*	↑		? —0.65±
		Varangian*		
		↑		? —0.69±
		Sturtian*		
		↑		? —0.75±
Adelaidean*	Karatani* (U. Riphean)			
		?		
				? —0.95±
	Yurmatin* (M. Riphean)			
				? —1.35±
	Burzyan* (L. Riphean)			
				? —1.5± 0.15
and others	and others	and others		

* Familiar names adapted to this scheme for illustration.

† Reference point in boundary stratotype.

Table 2 Geochronometric scale with Earth time divisions

Ga	'aetas'	'aevum'	Approximate position on chronostratigraphic scale
0	? Latest		Cenozoic and Mesozoic
	Late		
0.25		(Novum tempus)	
	Middle		Palaeozoic
-0.5		Novotemp. (?Novot.)	
	Early		
		(*Late PC)	Precambrian
-1.0	Late		
		(Medium tempus)	
-1.5		Mediotemp. (? Mediot.)	
	Middle		
-2.0		(*Middle PC)	
	Early		
-2.5	Late		
		(Antiquum tempus)	
-3.0		Antiquotemp. (? Antiquot.)	
	Middle		
-3.5		(*Early PC)	
	Early		
-4.0	Late		
		(Priscum tempus)	
-4.5	? Middle	Priscotemp. (? Priscot.)	
	? Early		

* Informal names and divisions of Precambrian time as proposed by IUGS Precambrian Subcommittee and borrowed here to show that the divisions should correspond to named divisions of the geochronometric scale. But the boundaries of these divisions have yet to be decided, and may not correspond to the values suggested here, in which case this scheme might be modified accordingly.

Precambrian divisions along these lines have been proposed¹¹⁻¹⁴. Indeed, the time is ripe, in connection with the standardisation of the initial Cambrian boundary¹⁵, similarly to define some earlier divisions¹⁶. These should not be defined in terms of general events (such as the occurrence of plant life or glaciation), even though that may provide hope of good correlation. Table 1 indicates how it could be achieved. It would be necessary to decide on a classification, a nomenclature and a standardisation (at reference points in selected boundary stratotypes). Table 1 illustrates these points.

New classification and nomenclature of the geochronometric scale

At the same time there is pressure from workers concerned with Precambrian geology to improve the geochronometric scale by defining named divisions longer than a million years. Here, again, it is necessary to decide on a classification, a nomenclature and a standardisation.

The example shown in Table 2, like that shown in Table 1 is designed rather to illustrate the general principle than to press its particular merits, so that out of criticism an agreed scale may emerge.

An hierarchy with divisions larger than a year would be useful as many events have ages which would be easier to express in divisions of a longer duration. I suggest two divisions. The larger are compounded of smaller divisions of 0.5 Ga ($=0.5 \times 10^9$ yr). I considered a scheme with divisions at -1.0, -2.0, -3.0, -4.0 Ga (that is, a gigennial system) but rejected it because it might have put into words a reversed system as confusing as centuries BC. I then tried a scheme with four names for spans of up to 1.5 Ga and a threefold subdivision of each—early, middle and late (or with subscripts 1, 2 and 3, as in Russian stratigraphic abbreviations).

I chose the division at -2.5 Ga because it is probably the

most popular point for dividing Precambrian time⁹. The 1.5 Ga division may be termed an *aevum* (eon) and the 0.5 Ga span an *aetas* (age).

Existing nomenclature is Precambrian-orientated, loaded with associations, and ambiguous or hybrid, so new terms are needed for the new task. They will need to be clear and convenient if they are to be used in preference to the obvious numerical alternative.

Most stratigraphic roots are derived from the Greek (chron, period, era, eon, eo, proto, archaeo, palaeo, meso, neo and kaino) possibly because they combine more elegantly than Latin roots. Latin has hardly been used so far, so I suggest that Greek be used with the chronostratigraphic scale and Latin be used with the geochronometric scale (with the exception of 'geochronometry'). I considered several Latin roots: *ortus* and *natus* (born); *origo* (origin); *priscus* (ancient); *matutinus* (early); *vetustus* and *antiquus* (old); *medius* (middle); *serus* (late) and *novus* (new). Words like *novus* may be combined in the substantial form *novum tempus* (novotime) and the adjectival form *novotemporalis* (novotemporal).

I suggest that for general international use the abbreviated form *novotemp* (or *novot*) be used. Many scientists casually use adjectives as nouns: thus, 'The Precambrian' is a common abbreviation for the Precambrian rocks, history, time, research, or whatever noun is appropriate in context. So in this way the single adjectival abbreviated form might serve as an international technical name without inflection.

The particular names suggested here are not necessary to the scheme and others may be devised. Greek roots are easier and if used with a distinctive suffix may be more acceptable if defined and agreed as a whole.

Standardisation will be simply in years and there is a consensus that very round figures be used so as to avoid any implication of definition by historical events.

Each subdivision (*aetas*) is defined as a precise periodic multiple of 500 Ma. Strictly, years BP go back from a datum at 1950 but it is suggested that, unless a subdivision Latest Novotime beginning at 1950 be used, Late Novotime be extended. Similarly, at the beginning of Priscotime a different procedure may be considered.

The numerical expression of the scale could be distinguished by the negative sign from the same value used as a duration, in preference to the use of BP (see ref. 17).

The magnitude of time span of the proposed divisions is suitable for general discussion of the physical and chemical evolution of the Earth and indeed of the Solar System, so providing a convenient planetary descriptive scale. Cosmologists may advise on how to define the early spans of Priscotime if it be desired to consign the early history of the Earth mostly to its third subdivision.

Scales independent of history

These suggestions distinguish clearly between the chronostratigraphic and geochronometric scales and also avoid the restriction of the latter to Precambrian time ('Precambrian' belongs to the former). Nevertheless, the geochronometric scale will serve Precambrian time with eight of its nine or ten subdivisions. If a hybrid division be required it can be expressed in hybrid form, namely, Precambrian Novotemp (or PC Novot.).

It is desirable to avoid names for the geochronometric scale that are associated with the confused history of Precambrian stratigraphy. There are, however, a few rather strong weeds in Rankama's "rock garden" that will be difficult to eradicate, namely: Archaeon, Proterozoic, and Eo-Cambrian. These may usefully continue without definition. There is an advantage in retaining a generally understood nomenclature which is intentionally vague so that when an uncertain age is expressed the old names can be used. The new scales would thereby be spared misuse.

In the meantime, if Early, Middle and Late Precambrian time be used as informal names (as suggested by Rankama through the Precambrian Subcommittee) the values chosen

for calibration should also be used for dividing Antiquot, Mediot, and Novot.

Finally, agreement on the two artificial scales suggested merely improves the equipment for dealing with the real problems of geology requiring the use of all kinds of phenomena for correlation, and describing their evolution on either scale. Phenomena also need some regulation as to their terminology and nomenclature but their history can never be regulated, and this is the fundamental distinction between the framework and phenomenon categories⁶. Both scales are needed to investigate geological history and to do so they should avoid dependence on it.

Professor C. O. Brink contributed the classical expertise in proposing the new Latin terminology.

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Ethidium bromide inhibits appearance of closed circular viral DNA and integration of virus-specific DNA in duck cells infected by avian sarcoma virus

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The DNA of avian sarcoma virus assumes a closed circular configuration before integration into the host cell chromosomal DNA. Ethidium bromide reduces the formation of superhelical viral DNA and concurrently blocks integration of the viral genome. Inhibition of integration of viral DNA results in the inhibition of virus replication.

FOLLOWING infection of permissive cells by RNA tumour viruses, a DNA intermediate is transcribed from viral RNA (ref. 1) and subsequently integrated into the host cell genome². Although synthesis of viral DNA seems to be crucial in the virus life cycle, it has not been established whether virus production depends on integration of viral DNA. Since no viral mutants defective in integration have been isolated, alternative approaches have been sought to document the necessity of integration for virus replication. One such approach is to block integration with chemical inhibitors. We show here that ethidium bromide (EB), a phenanthridine dye, allows synthesis of normal quantities of viral DNA but blocks its integration into the host DNA, probably by interfering with the formation of covalently closed circular DNA. This closed circular molecule, which can be demonstrated in alkaline sucrose and equilibrium density gradients, is presumably the form of viral DNA just before its integration. We also present evidence for a direct relationship between the amount of viral DNA integrated and the amount of virus-specific RNA produced by the infected cell.

EB affects viral DNA integration

Peking duck cells were pretreated for 6 h with EB (1.0 $\mu\text{g ml}^{-1}$) and then infected with B77 avian sarcoma virus in the presence of the inhibitor. Forty hours after infection, DNA was isolated from untreated (control) and EB-treated cells and assayed for virus-specific nucleotide sequences by testing its capacity to influence the reassociation of double-stranded viral DNA synthesised *in vitro*³. In the experiment presented in Fig. 1a

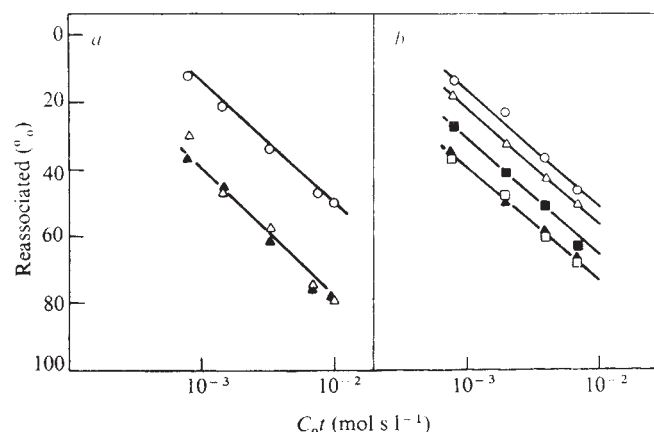


Fig. 1 Effect of EB on integration of viral DNA. Peking duck cells (prepared from 12–14-d-old embryonated eggs) were grown to a density of $4\text{--}5 \times 10^6$ per 100 mm plate. Half of the cells were infected with B77 virus at a multiplicity of infection of 3 in the presence of polybrene ($2 \mu\text{g ml}^{-1}$) and the other half was pretreated for 8 h with EB ($1.0 \mu\text{g ml}^{-1}$) and then infected in the presence of the inhibitor. After 40 h incubation, high molecular weight DNA was extracted and tested for integration as described by Varmus *et al.*³. To detect the presence of virus-specific nucleotide sequences, radioactive duplex virus-specific DNA (representing about 30% of the total viral genome²) was denatured and reassociated in the presence of various samples of cellular DNA. Acceleration of the reassociation of the radioactive DNA indicates the presence of virus-specific nucleotide sequences in the cellular DNAs⁸. Each reaction mixture contained radioactive DNA (1.6 ng ml^{-1} , $15,000 \text{ c.p.m. ng}^{-1}$). Reassociation was monitored by fractionation on hydroxyapatite. *a*, : (○) normal duck DNA (1.6 mg ml^{-1}); DNA (△) from B77-infected control (1.6 mg ml^{-1}) or (▲) EB-treated (1.5 mg ml^{-1}) duck cells. *b*: (○) normal duck DNA (1.6 mg ml^{-1}); (□) network DNA (1.3 mg ml^{-1}) or (■) supernatant DNA (1.1 mg ml^{-1}) from control cells or (△) network DNA (1.3 mg ml^{-1}) or (▲) supernatant DNA (0.5 mg ml^{-1}) from EB-treated cells.

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Table 1 Inhibition of integration by EB

Sample	Total DNA		Network DNA		Supernatant DNA	
	$C_{0t_{1/2}}$	copies per cell	$C_{0t_{1/2}}$	copies per cell	$C_{0t_{1/2}}$	copies per cell
Uninfected	1.0×10^{-2}	0.00	9.2×10^{-3}	0.00	9.2×10^{-3}	0.00
Infected untreated	1.7×10^{-3}	1.20	2.0×10^{-3}	1.10	3.7×10^{-3}	0.60
EB-treated	1.7×10^{-3}	1.20	7.0×10^{-3}	0.15	2.0×10^{-3}	2.80

$C_{0t_{1/2}}$ values were obtained from reassociation kinetics given in Fig. 1. Copy numbers were computed from $C_{0t_{1/2}}$ as described by Gelb *et al.*¹⁷.

and Table 1, 1.2 copies per cell of viral DNA were synthesised in either the presence or absence of EB, indicating that EB has no effect on the net synthesis of viral DNA. In some experiments (data not shown), 15–20% inhibition was observed with this dose of EB. Since virtually normal levels of viral DNA are synthesised, these data indicate that, in contrast to the results of studies *in vitro*⁴, EB does not inhibit the action of reverse transcriptase in the infected cell.

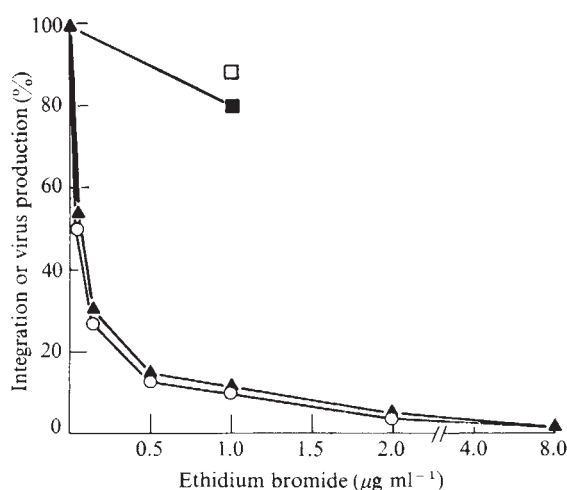


Fig. 2 Duck cells, either untreated or pretreated for 8 h with EB, were infected with B77 virus at a multiplicity of infection of 5 in the absence or presence of the inhibitor. After 38 h, high molecular weight DNA was isolated and assayed for integration as in Fig. 1. Also, one plate from each batch was labelled with ^3H -uridine ($16 \mu\text{Ci ml}^{-1}$; 27 Ci mmol^{-1}), and fluids were collected and banded in equilibrium sucrose gradients as described in the legend to Table 1. One plate from duck cells infected 7 d previously was labelled similarly after 24 h (□) or 48 h (■) treatment with EB. ○, Integration of viral DNA; ▲, virus production.

Integration of viral DNA into the cell genome was, however, affected by EB. The DNA was tested for integration by a recently described technique². Under appropriate conditions, reiterated cell DNA reassociates to form network-like structures; viral DNA covalently linked to strands of cell DNA appears in these structures. Network DNA from cells infected in the absence of EB accelerated the rate of reassociation of

labelled viral DNA by a factor of 5, whereas the network DNA from EB-treated cells had only a marginal influence on the reassociation (Fig. 1b). The data indicate that less than 15% of viral DNA (0.15 copy compared with 1.1 copies in the control) was integrated in the presence of EB (Table 1). As expected, DNA from EB-treated cells that had not entered into networks markedly decreased the $C_{0t_{1/2}}$ of labelled viral DNA, indicating considerable (six- to eightfold) enrichment of virus-specific sequences in the supernatant which included all unintegrated viral DNA. From these results we conclude that EB does not inhibit viral DNA synthesis, but prevents integration of viral DNA into host cell DNA.

Similar results were obtained with EB ($0.5 \mu\text{g ml}^{-1}$) but with still lower concentrations, the extent of inhibition decreased significantly (Fig. 2). Concentrations of EB above $1.0 \mu\text{g ml}^{-1}$ not only blocked integration but also suppressed synthesis of viral DNA. For example, in the presence of EB at $2.0 \mu\text{g ml}^{-1}$ only 45% of control levels of viral DNA was synthesised, and integration was totally inhibited. The suppression of viral DNA synthesis by the higher doses of EB could be caused by cytotoxic effects of the drug. Doses of $1.0 \mu\text{g ml}^{-1}$ or less had little or no effect on cell growth during a 48-h exposure (see below, Table 2), whereas higher doses severely depress cell growth (R.V.G., B.W.J.M., J.M.B., and H.E.V., unpublished).

EB and supercoiled viral DNA

Recently, we have shown that at least 10–20% of viral DNA exists in a covalently closed duplex circular form 8–10 h after infection of duck cells by avian sarcoma virus^{5,6}. Since EB binds to duplex DNA and causes unwinding of the molecule, we proposed that EB might intercalate with double-stranded, viral DNA to prevent or reverse the formation of super-coiled DNA (form I) and thereby inhibit integration. The following evidence supports this notion.

Integration of viral DNA into the cell genome starts about 10 h after infection². If formation of supercoiled viral DNA is required for integration, we expect maximum levels of this species before integration. Therefore, untreated and EB-treated duck embryo fibroblasts were infected for 9 h in the absence and presence of the dye and cells were fractionated according to the procedure described by Hirt, which has been used to separate papovavirus DNA from host cell DNA⁷. The DNA in the Hirt supernatant was analysed for the presence of closed circles in an alkaline sucrose gradient. Three distinct peaks of viral DNA, detected by hybridisation to cDNA (DNA complementary to viral genome), were observed in both control and EB-treated supernatant fractions. About 18% of the

Table 2 Effect of EB on virus production in B77-infected duck cells

	Cells per 100-mm plate	Viral DNA copies per cell	Integration (%)	Virus production	
				^3H -uridine incorporation (c.p.m.)	FFU ml^{-1}
Control	8.7×10^6	1.7	100	3,095	7.5×10^4
EB	7.2×10^6	1.5	17	417	8.5×10^3

Duck cells (4×10^6 per 100 mm plate), untreated or pretreated for 4 h with EB ($1.0 \mu\text{g ml}^{-1}$) were infected with B77 virus at a multiplicity of infection of 5 for 40 h in the absence or presence of EB. High molecular weight DNA was isolated and assayed for integration as described in the legend to Fig. 1. One plate from each group was labelled with ^3H -uridine ($8 \mu\text{Ci ml}^{-1}$; 27 Ci mmol^{-1}) for 8 h and the growth fluids were banded in an equilibrium sucrose gradient (15–55% sucrose in 0.1 M NaCl, 10 mM Tris-HCl, pH 7.4, 10 mM EDTA; SW41 rotor, 3.5 h at 40,000 r.p.m. and 4°C). The tritium label banding at a density of $1.15\text{--}1.17 \text{ g ml}^{-1}$ was taken as the amount incorporated into the virion RNA. Aliquots of growth fluids from control and EB-treated samples were also assayed for virus production by focus formation on chicken cells. FFU, Focus-forming units.

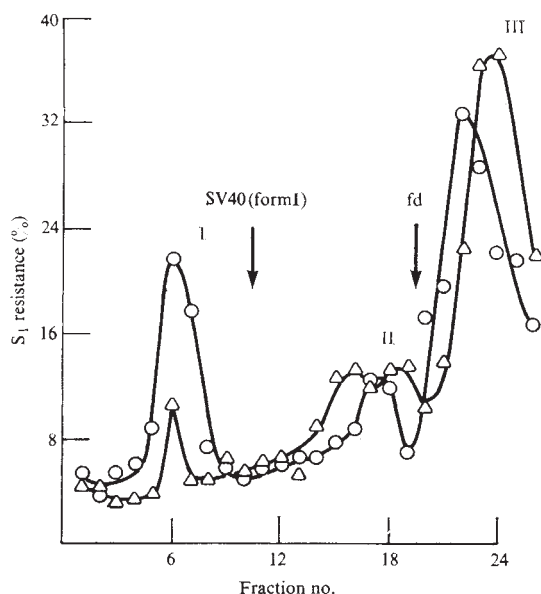


Fig. 3 Sedimentation of viral DNA in alkaline sucrose gradient. Duck cells, either untreated or pretreated with EB ($0.25 \mu\text{g ml}^{-1}$) for 14 h, were infected with B77 virus at a multiplicity of infection of 4–5 in the absence or presence of EB ($1.0 \mu\text{g ml}^{-1}$). After 9 h, cells were collected and fractionated as described by Hirt⁷. DNA was isolated from the Hirt supernatant by treatment with pronase followed by phenol extraction. After ethanol precipitation, the DNA was treated with pancreatic RNase in 10 mM Tris-HCl (pH 7.4)–10 mM EDTA, re-extracted with phenol, ethanol precipitated and analysed on a 5–20% sucrose gradient in 0.3 M NaOH, 0.7 M NaCl and 1 mM EDTA. Centrifugation was for 3 h in a SW41 rotor at 40,000 r.p.m. and 20°C . Fractions were collected from the bottom of the gradient, supplemented with calf thymus DNA ($50 \mu\text{g ml}^{-1}$) to serve as carrier, and then heated for 2 h at 80°C to reduce the size of DNA. The fractions were neutralised and precipitated with ethyl alcohol, DNA was collected by centrifugation and assayed for virus-specific sequences using ^3H -labelled cDNA (1,270 c.p.m. per fraction; $20,000 \text{ c.p.m. ng}^{-1}$). Annealing was for 4 d at 68°C in 0.6 M NaCl in 40 μl volume. Resistance of ^3H -cDNA to S1 nuclease was determined as described previously¹⁸. Under these conditions of annealing, the reaction was linear from 0.05 to 0.5 ng (5–30% S_1 resistance) of viral DNA¹⁶. This permits quantitation of virus specific DNA in an individual sample. Supercoiled SV40 (form I, 53S) and single-stranded circular fd DNA (20S) were run in parallel gradients. Sedimentation is from right to left. $\circ$, Control; $\triangle$, EB.

virus-specific DNA sedimented at 62–65S (peak I, Fig. 3); this species represents supercoiled viral DNA^{5,6} (R.V.G. *et al.*, unpublished). More than 60% of the viral DNA is much smaller in size (molecular weight $< 1.0 \times 10^6$; peak III). About 10–15% sedimented at approximately 22S, corresponding to a molecular weight of 3×10^6 to 3.5×10^6 (equivalent to the size of the 35S subunits of the viral genome¹). EB suppressed the formation of closed circles (peak I) by a factor of 4–5 without affecting the other forms (peaks II and III; Fig. 3); less than 0.1 ng out of 2.8 ng was found as supercoiled DNA in the EB-treated sample compared with 0.5 ng out of 2.4 ng in the control (see legend to Fig. 3). These results suggest that EB specifically blocks or reverses the formation of supercoils. Further evidence in support of this comes from the following experiment.

Supercoiled DNA (form I) binds less EB than open circular (form II) or linear (form III) duplex DNA⁸. Consequently, form I DNA bands at a higher buoyant density (about 0.035 g ml^{-1} greater than forms II and III) in CsCl-EB equilibrium density gradients⁸. Analysis of the Hirt supernatant fraction from duck cells infected for 9 h with B77 virus revealed that two separate peaks of viral DNA could be resolved with a density difference of about 0.036 g ml^{-1} ; under similar conditions

SV40 forms I and II were, as expected, separated by a density difference of 0.038 g ml^{-1} . The hybridised viral DNA banding at higher density is consistent with a closed circular molecule.

In agreement with the results shown in Fig. 3, EB suppressed the formation of only the supercoiled DNA by more than 80% (Fig. 4). Computation of the amount of viral DNA from percentage hybridisation¹⁶ indicates that comparable amounts of viral DNA were synthesised in EB-treated and untreated cells (about 6 ng), but less than 0.2 ng of the total banding as form I in the EB-treated sample as opposed to about 1.3 ng in the control.

To demonstrate unequivocally that the DNA binding at the higher density in Fig. 4 is closed circular DNA, we showed that its density in CsCl-EB gradients could be altered by limited exposure to pancreatic DNase. Viral DNA from the Hirt supernatant of cells infected for 9 h was centrifuged to equilibrium in a CsCl-EB gradient; 15–20% of the total viral DNA cosedimented with PML21 form I (Fig. 5a). When single-strand breaks were introduced by pancreatic DNase, both the

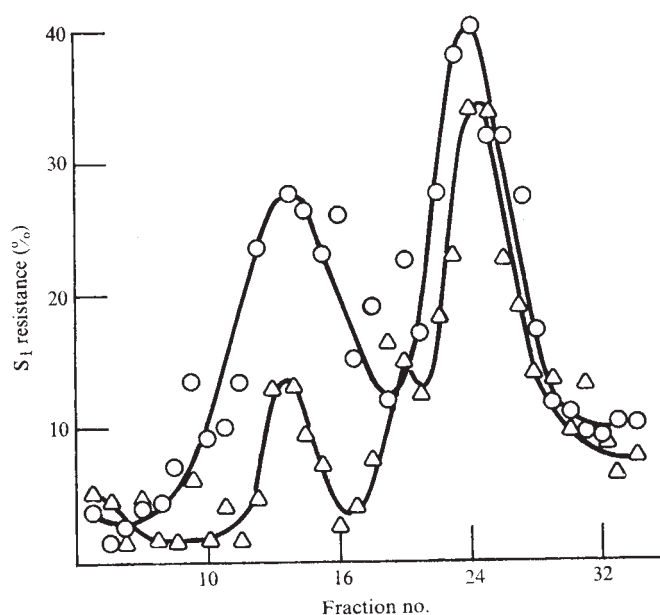


Fig. 4 Equilibrium gradient centrifugation of viral DNA. The remaining portions of the Hirt supernatants from duck cells infected with B77 virus in the presence or absence of EB (see legend to Fig. 3) were analysed in a CsCl-EB gradient. To a 6.6-ml sample in 10 mM Tris-HCl–3 mM EDTA 6.3g, CsCl and EB ($300 \mu\text{g ml}^{-1}$) were added and centrifuged for 66 h in a type 40 rotor at 33,000 r.p.m. and 20°C . ^3H -labelled SV40 DNA was also centrifuged in a parallel gradient. Fractions were collected and assayed for virus-specific DNA with ^3H -cDNA (1,330 c.p.m.; $20,000 \text{ c.p.m. ng}^{-1}$) as described in Fig. 3. The viral DNAs corresponding to supercoiled and open or linear forms banded at 1.594 and 1.558 g ml^{-1} respectively. SV40 form I has a buoyant density of 1.590 g ml^{-1} under similar conditions and banded between fractions 8 and 14. $\circ$, Control; $\triangle$, EB.

viral DNA and PML21 marker DNA shifted to a lighter density peak (Fig. 5c), corresponding to form II or III. Re-banding of the same material without DNase treatment showed that form I DNA was still intact (Fig. 5b). This effect of pancreatic DNase on the density shift of viral DNA strongly supports our conclusion that at least a portion of the viral DNA synthesised *in vivo* exists as a closed circular molecule.

We have consistently observed that a measurable amount of viral DNA bands at a position intermediate to the heavy and light density peaks of DNA, thereby broadening the bandwidth of form I DNA (Figs 4 and 5). The density pattern of this DNA is unchanged by treatment with pancreatic DNase. The nature of this species is at present unclear and is being investigated.

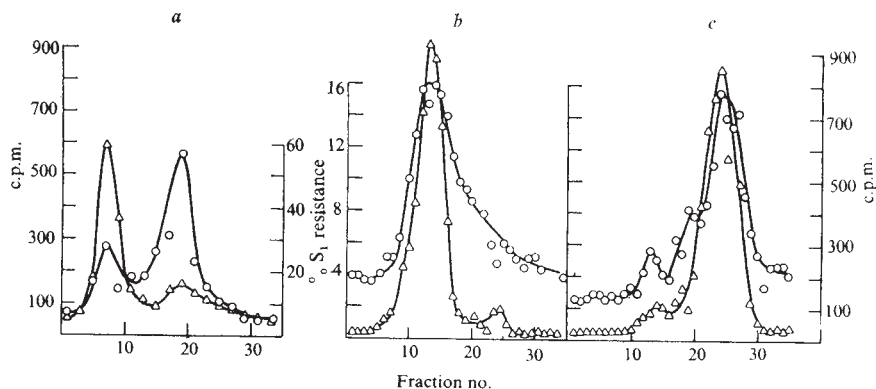


Fig. 5 Conversion of form I DNA to form II by pancreatic DNase. Duck cells were infected with B77 virus at a multiplicity of infection of 6 and after 9 h, cells were collected and fractionated as described by Hirt⁷. DNA was isolated from the Hirt supernatant as described in the legend to Fig. 3. ³H-labelled PML21 plasmid DNA (molecular weight 7.1×10^6 dalton) was added to the supernatant DNA and banded in an equilibrium CsCl-EB gradient (6.3 g CsCl in 6.8 ml sample containing EB at $300 \mu\text{g ml}^{-1}$). *a*, One-fourth volume from alternate fractions was hybridised to ³²P-labelled cDNA as in Fig. 3. The DNA banding at heavy density (fractions 4–12) was pooled and EB was removed by passing through Dowex-AG50. Calf thymus

DNA ($60 \mu\text{g}$) was added and the pooled sample was dialysed against 20 mM Tris-HCl to remove CsCl. The sample was divided into two equal portions; one portion served as control (*b*) and the other half was treated with pancreatic DNase (*c*) (Worthington) in 10 mM MgCl₂, 20 mM Tris-HCl, pH 7.4, 10 mM NaCl, bovine serum albumin ($30 \mu\text{g ml}^{-1}$) and 5×10^{-6} μg DNase for 20 min at room temperature. The reaction was stopped by the addition of 50 mM EDTA and the samples were centrifuged to equilibrium in CsCl containing EB ($300 \mu\text{g ml}^{-1}$). The conditions of centrifugation and hybridisation were as described in Fig. 4. $\circ$, Percentage hybridisation to ³²P-cDNA; $\triangle$, ³H-labelled PML21.

EB inhibits virus production

To explore the relationship between integration of viral DNA and virus production, the following experiments were performed. Cells were pretreated with $1.0 \mu\text{g ml}^{-1}$ EB for 6 h and infected for 40 h in its presence. DNA was then isolated and tested for the presence and integration of virus-specific nucleotide sequences. Virus production was monitored by the incorporation of ³H-uridine into the RNA of mature virus released into the medium and by assay of the culture medium for focus-forming activity. In addition, the rate of synthesis and concentration of virus-specific RNA in the infected cells were measured by hybridisation of cellular RNA to virus-specific complementary DNA (cDNA) synthesised *in vitro*⁹. The results from a typical experiment (Table 2) show that EB ($1.0 \mu\text{g ml}^{-1}$) suppressed integration and virus production coordinately by a factor of 6–8. Measurement of virus-specific RNA in the EB-treated cells by hybridisation to ³H-labelled cDNA gave similar results (Table 3). EB at $1 \mu\text{g ml}^{-1}$ reduced the concentration of viral RNA by about 80% as early as 24 h after infection, and the degree of inhibition did not change appreciably during 72 h. Substantial amounts of input viral RNA, however, result in high levels of background and thus might obscure the total effect of EB on viral DNA synthesis in this type of experiment. This problem can be overcome by studying the effect of EB on the *de novo* synthesis of virus-specific RNA. Therefore, 48 h after infection, cells were pulse-labelled with ³H-uridine, and RNA was isolated and analysed for virus-specific sequences by hybridisation to

unlabelled cDNA as described previously¹⁰. In these conditions EB inhibited synthesis of virus-specific RNA by approximately 75% (Table 4), the reduction being proportional to the reduction in the amount of viral DNA integrated into cell DNA.

The data in Fig. 2 further substantiate the conclusion that virus production depends on integration of viral DNA. There is a good correlation between the extent of viral DNA integrated and the magnitude of virus production. The inhibition of viral replication is not a simple consequence of the effect of EB on cell growth for there was only a barely perceptible decrease in the number of cells (Table 2). Moreover, the effect of EB on virus production is appreciable only if the inhibitor is added before integration. Addition of the dye ($1.0 \mu\text{g ml}^{-1}$) to cells after 24 h of infection (data not shown) or to fully infected cells producing virus (Fig. 2) did not inhibit virus production. These results strongly suggest that integration of viral DNA into the cell genome is mandatory for virus production.

Our results are different from those obtained by Reichert and Hare¹¹, who demonstrated that virus replication is unaffected by EB at $1.0 \mu\text{g ml}^{-1}$. Substantial differences in the experimental protocols used might partly explain this dissimilarity. Bader¹², on the other hand, found that this concentration of EB reduced viral RNA synthesis by 60% either in JSL-V5 cells infected by the Rauscher strain of murine leukaemia virus or in chick embryo fibroblasts infected by Rous sarcoma virus. We observed similar inhibition of virus production in chick cells (70%) as opposed to duck cells (80–85%) in the presence of EB ($1.0 \mu\text{g ml}^{-1}$).

Table 3 Synthesis of B77 viral RNA as analysed by hybridisation to cDNA

Time after infection (h)	Integration (%)		$C_{t1/2}$ (mol s ⁻¹)		Virus-specific RNA (%)		Inhibition (%)
	Control	EB	Control	EB	Control	EB	
24	—	—	5.5×10^2	2.5×10^3	0.004	0.0008	78
48	100	15	2.4×10^2	1.3×10^3	0.009	0.0015	84
72	—	—	1.1×10^2	6.0×10^2	0.020	0.0030	82

Duck cells were pretreated with EB ($1.0 \mu\text{g ml}^{-1}$) for 2 h and infected with B77 virus in the presence of the inhibitor. Control cells did not receive EB. At the indicated times, cells were collected and RNA was extracted with SDS-pronase-phenol¹⁸. The RNA was then hybridised to ³H-labelled cDNA synthesised *in vitro*. Details of this technique were published elsewhere¹⁸. DNA was tested for integration as described in the legend to Fig. 1. The amount of virus-specific sequences in the total cell RNA was calculated from the $C_{t1/2}$ (2×10^{-2}) obtained with purified 70S virion RNA and cDNA¹⁸. Avian sarcoma virus-transformed cell RNA has a $C_{t1/2}$ ranging from 2 to 10 suggesting that 0.2–1% of the total RNA is virus-specific.

Table 4 Effect of EB on virus-specific RNA

Sample	Hybridisation input		Radioactivity in hybrid (%) [‡]		Virus-specific RNA (%) [§]
	³ H*	³² P†	³ H	³² P	
Control	470,655	1042	0.28	61.0	0.46
EB-treated	373,291	1169	0.08	65.0	0.12

*Duck embryo cell monolayers were pulse-labelled for 100 min with ³H-uridine (1 mCi ml⁻¹ in nucleoside-free medium) 48 h after infection with B77 virus in the presence or absence of EB (1.0 µg ml⁻¹). At the conclusion of the pulse, total cell RNA was extracted as described previously¹⁸. The specific activity of the purified RNA was 1.0 × 10⁶ c.p.m. µg⁻¹.

†³²P-labelled 70S RNA (specific activity 1 × 10⁶ c.p.m. µg⁻¹) was purified from B77 virus⁹.

‡The hybridisation mixture in a total volume of 10 µl 2 × SSC (NaCl (0.15M)—Na citrate (0.015M)) contained the indicated amounts of ³H-cell RNA and ³²P-70S B77 RNA plus 24 µg yeast RNA and either 30 ng unlabelled cDNA synthesised using Prague-C Rous sarcoma virus or 30 ng phage λ DNA as control. After annealing for 24 h at 68°C, the percentage of RNA present in RNA-DNA hybrids was determined essentially as described by Parsons *et al.*¹⁰. Results were corrected for a background of 0.3% in control samples.

§Calculated as ³H hybridised (%) / ³²P hybridised (%) × 100.

The claims that virus production occurs in mitochondria^{13,14} have been challenged¹². Since EB selectivity inhibits mitochondrial RNA transcription and DNA replication¹⁵, our results could be interpreted to mean that virus production occurs in mitochondria, or that a function involved in mitochondrial DNA replication also participates in viral DNA replication and integration. This seems unlikely, however, for two reasons: (1) although viral DNA synthesis occurs in the cytoplasm shortly after infection, virus-specific DNA subsequently migrates into the nucleus and integrates with chromosomal DNA¹⁶, and (2) mitochondrial DNA, isolated from B77-transformed duck cells, did not hybridise to virus-specific ³H-labelled cDNA (R.V.G. unpublished). These results argue against the possibility that mitochondria are involved in virus replication and support Bader's results¹².

Molecular events before viral DNA integration

We have presented evidence favouring the following scheme for the synthesis of viral DNA after infection by avian sarcoma viruses. Viral DNA is made in the cytoplasm during the first 6 h after infection as a linear (or open circular) duplex molecule of a length similar to that of a subunit of the viral RNA genome (molecular weight 3.0 × 10⁶)^{5,6,16}; these molecules are unusual in that the predominant form contains full length strands complementary to the viral genome ('minus' strand) but relatively short 'plus' strands of DNA²⁰ (less than 1.0 × 10⁶ daltons; H.E.V. *et al.*, in preparation). Viral DNA then migrates into the nucleus at about the time that we have observed the supercoiled form of viral DNA¹⁶. Under ordinary circumstances, all or a substantial portion of viral DNA becomes integrated into the cell genome between 9 and 24 h after infection². The

presence of EB suppresses the accumulation of closed circular viral DNA, presumably by intercalation into DNA duplexes. Since the accumulation of closed circles and the integration of viral DNA are reduced concomitantly and to similar extents, we propose that a circular molecule is the form which undergoes the crossing-over step(s) required for integration. If our hypothesis is correct, the mechanism of integration for RNA tumour viruses is fundamentally similar to that for several DNA tumour viruses¹⁹. The molecular mechanism by which circles are formed remains uncertain.

Since viral RNA and progeny virus are not made when integration of viral DNA is blocked by EB, it seems that integration is a mandatory step for full expression of the viral genome. We cannot as yet exclude the possibility that the drug also exerted subtle effects on cell metabolism unrelated to viral events.

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Note added in proof: Closed circular viral DNA has also been identified in mouse cells infected by murine leukaemia virus (Gianni, A. M., Smotkin, D., and Weinberg, R. A., *Proc. natn. Acad. Sci.*, (in the press)).

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letters to nature

On the planetary theory of sunspots

It has been proposed^{1,2} that sunspot activity is affected by positions of the planets, and calculations have been presented³, which purport to show that planetary tides on the Sun vary in the same way as the sunspot variations. We believe that the apparent agreement of the sunspot cycle with planetary tidal effects is an artefact of the calculation.

The calculation in question³ was used to compute the absolute value of the difference in tidal potential between Earth-Venus

conjunctions and oppositions at the sub-Jupiter solar point. The effect of Mercury, one of the strongest tide-raising planets was ignored on the basis that its period is short compared with that of sunspot activity. The absolute value of the tide and the effect of partial line-ups of Venus (or the Earth) with Jupiter were not computed. Furthermore, it is not clear that the absolute difference between opposition and conjunction tidal potentials has any physical meaning.

Here we compute the full tidal problem for Mercury, Venus, Earth and Jupiter, the tide-raising planets, taking into account

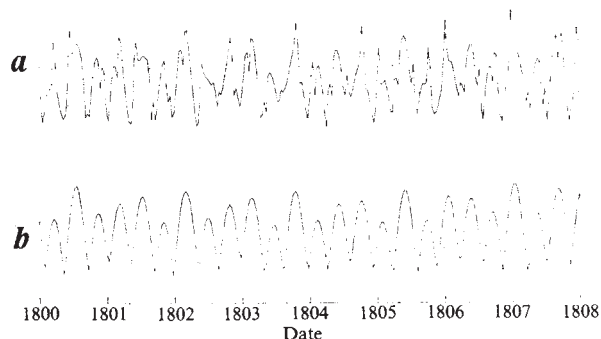


Fig. 1 Tidal potential for the years 1800–08. *a*, Full four-planet tidal potential; *b*, tidal potential excluding Mercury. Both scales are identical.

the complete orbital elements, including eccentricity, inclination and their variation with time.

At any given time, the tidal potential at a given point *M* on the surface of the Sun caused by the planets is proportional to

$$T = \sum \frac{m_i}{d_i^3} (\cos^2 z_i - \frac{1}{3})$$

where m_i is the mass of the planet, d_i its distance from the centre of the Sun and z_i the angle from the planet to point *M* as seen from the centre of the Sun. We restrict ourselves to Mercury, Venus, Earth and Jupiter. Mars, Saturn and the other outer planets can easily be shown to have trivial contributions compared to the above planets.

Table 1 Comparison of tidal and sunspot dates

Tidal peak	Sunspot peak	Dts*
1809	1816	–7
1822	1830	–8
1833	1837	–4
1845	1848	–3
1857	1860	–3
1869	1871	–2
1881	1884	–3
1892	1894	–2
1905	1906	–1
1916	1918	–2
1927	1928	–1
1939	1938	+1
1951	1948	+3
1963	1958	+5
1974	1969	+5

* Dts is the difference between tidal and sunspot peaks in years.

Tidal peaks are taken from Fig. 2 (and its continuation up to the year 2000). Sunspot peaks are from Wood.

In the plane of the ecliptic, the potential depends on the longitude ϕ through a first order polynomial of $\sin 2\phi$ and $\cos 2\phi$. Over a period of 10 d (our sampling interval) the Sun rotates about halfway around its axis and therefore any given point attached to its surface is subject to the whole variation of the tidal potential. So we characterised our problem by the maximum value (over ϕ) of this polynomial. We note that Mercury is the slowest planet around the Sun relative to a point on the Sun's surface and its contribution, contrary to previous arguments³ should therefore not be neglected.

The positions of the planets were computed from the best available planetary elements⁴ for 65,536 epochs at intervals of 10 d, or roughly 1,800 yr, starting in the year 1800. A fast fourier transform (FFT) technique was then used to extract the power spectrum for comparison with the solar activity spectrum.

Figure 1 shows the tidal potential as a function of time for the years 1800–1808, both including Mercury (upper trace) and excluding it (lower trace). The high frequency effect is due to the eccentricity of Mercury's orbit and has a period of 0.24 yr.

Figure 2, an extension of the lower trace in Fig. 1, shows the tidal potential for the 25-yr period 1800–1825, excluding Mercury. The long period, beat-type phenomenon (~ 11.9 yr) arises because of the eccentricity of Jupiter's orbit. By contrast with the sunspot cycle, the tidal pattern repeats almost exactly every 11.9 yr and shows no evidence of a beat of ~ 100 yr; successive peaks in the tidal envelope are of almost exactly the same amplitude. Wood's samples³ (● in Fig. 2) have a spacing too large to provide a valid description of the tidal potential even excluding Mercury; such a sampling leads to aliasing of the lower frequencies. Figure 3 shows the power spectrum obtained from an FFT analysis of the whole 1,800-yr four-planet potential. The fundamental periods are those of the alignments of pairs of planets. Alignments of three or four planets come as beats of these primary values and consequently do not appear on the spectrum. The periods of Jupiter and Mercury and some of their harmonics show up because of the eccentricity of their orbits. The lower frequency part of the spectrum is very flat, Jupiter's being the longest period involved.

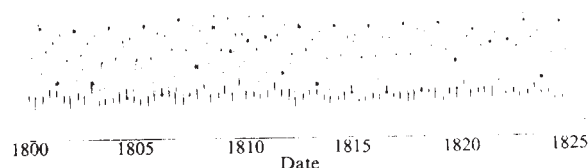
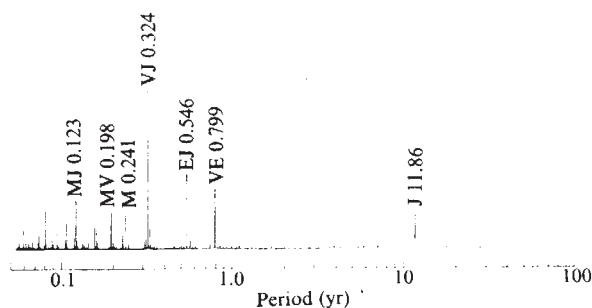


Fig. 2 Three-planet tidal potential for the years 1800–1825 (excluding Mercury). The marks (●) show the points used by Wood³.

Figure 4 (frequency scaled) shows an enlargement of the lower frequency part of the spectrum, superimposed with Cohen and Lintz's sunspot spectrum⁵. They showed a strong peak occurring at 11 yr, the familiar sunspot cycle, and smaller peaks at about 9.8, 95.8 and 8.3 yr. In addition they demonstrated that the longer period, ~ 180 -yr cycle proposed for the solar sunspot spectrum arises from the beat of the 11 and 9.8-yr cycles and is not an intrinsic periodicity. This removes the basis for one of the other planetary theories of sunspots⁶. Figure 4 shows no planetary peaks at 8.3, 9.8 or 95.8 yr, peaks which are prominent in the sunspot spectrum⁵.

The origin of the 11.08-yr tidal period claimed by Wood³ is not clear. Such a peak does not appear in our spectrum. In Wood's simplified three-planet system, it could only be the consequence of a Jupiter–Earth–Venus alignment. But if 11.08 yr is indeed a multiple of the Venus–Earth 0.799-yr alignment period, it is not a multiple of the Jupiter–Earth synodic period, and therefore is not a fundamental period of the problem. This discrepancy between the tidal and the sunspot activity periods is further demonstrated in Table 1, which gives sunspot peak dates³ and approximate dates of envelope maxima of the tidal potential, excluding Mercury. The average period between envelope peaks

Fig. 3 Power spectrum of tidal potential. The horizontal axis is scaled as $\log T$. The labels on the larger peaks identify the periods (yr) of alignments of planets (two-letter label) or the period of the planets themselves (one-letter label).



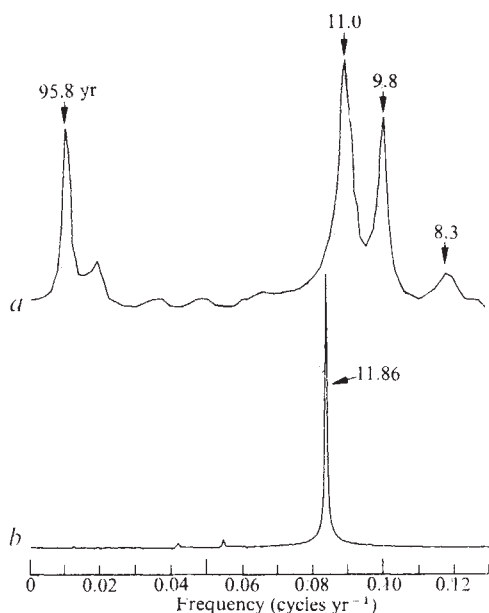


Fig. 4 Comparison of the lower frequency part of tidal spectrum (b) and sunspot spectrum (a, from ref. 5).

is 11.8 yr, the orbital period of Jupiter. From the beginning of the nineteenth century to the present the discrepancy between tidal peak dates and sunspot peak dates has slipped from approximately -7 yr to $+5$ yr. This is the difference between the 11.86-yr Jupiter period and the average sunspot period, 11.05 yr taken over 165 yr (14 cycles). Over a limited period of time, such as 1892 to 1939, the peak years agree to within a year or two but this is to be expected when comparing two periodic functions of nearly the same period. The next tidal envelope maxima occur in 1987 and 1998. In the incomplete tidal theory³ maxima are predicted for 1982, 1993 and 2003.

A further look at our tidal potential values shows no drastic effect expected in 1982 when planets are supposed to align on the same side of the Sun (see ref. 7). Indeed, better alignment will be achieved in 1990. Even then, no special tidal effect occurs because alignment of the outer planets has no pronounced effects on the tides. Alignment of the tide-raising planets within 10 degrees is a common phenomenon, occurring approximately every 10.4 yr and is not associated with drastic tidal effects.

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The solar spectrum at 8 mm

THE brightness temperature of the quiet Sun has been measured over almost the complete radio spectrum. It ranges from approximately 6,000 K at millimetre wavelengths to over 10^6 K at metre wavelengths. Various compilations of the disk temperatures^{1–3} had at centimetre and millimetre wavelengths from 10 to 100 GHz ($\lambda=3$ cm to 3 mm) (Fig. 1). A monotonically decreasing brightness temperature

presented by Shimabukuro and Stacey¹ from a model by van de Hulst⁴ is also shown in Fig. 1. Zheleznyakov⁵ has pointed out that the apparent dip in the observed disk temperatures near 50 GHz and at the apparent peak near 70 GHz, if real, would indicate an effective temperature inversion in the solar chromosphere from 3,000 to 3,300 km above the photosphere. Reber³, however, measured the slope of the brightness against frequency curve at 50 GHz and found that the disk temperature is, in fact, decreasing with increasing frequency in nearly perfect agreement with the van de Hulst⁴ model. Reber's method does not depend on an absolute measurement of the brightness temperature but rather on a relative measurement between two closely spaced frequencies, with a single instrument, thereby giving the slope with more accuracy than can be obtained by comparing a number of independent, absolute measurements.

We have measured the slope of the brightness–frequency curve near 36 GHz ($\lambda=8$ mm) to determine whether the slope best matches the van de Hulst model or the observations.

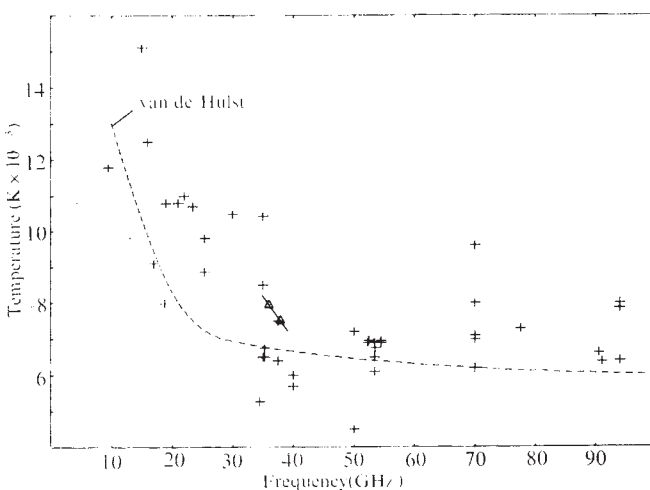


Fig. 1 Solar brightness temperatures against frequency. Triangles represent the measured slope of the curve between 36 GHz and 38 GHz.

The observational method used was similar to that of Reber's³ in which the new Moon is used as a calibration source. The ratio of the brightness temperatures of the Sun and Moon is given by

$$\frac{T_s}{T_M} = \left(\frac{T_A(\text{Sun}) - T_A(\text{sky})}{T_A(\text{Moon}) - T_A(\text{sky})} \right) \quad (1)$$

where T_M = brightness temperature of the Moon; T_s = brightness temperature of the Sun; T_A = measured antenna temperature of the Sun, Moon or sky, provided that the solid angle of the antenna beam is smaller than that of the Sun or Moon and that all measurements are reduced to the same zenith angle. The Sun–Moon ratio is independent of any knowledge of the antenna gain, atmospheric absorption and re-emission or radiometer calibration constants, provided that those parameters remain constant over an observation.

The ratio T_s/T_M was measured on 21 d within 3 d of new Moon at 36.0 GHz and 37.75 GHz, with a Dicke switched, superheterodyne radiometer. The half power beamwidth was $14'$. Two local oscillators were installed in the radiometer so that the frequency could be switched immediately from 36 GHz to 37.7 GHz. The Sun–new Moon ratios at the two frequencies were found to be 42.6 ± 0.5 at 36 GHz, and 40.6 ± 0.7 at 37.7 GHz.

Using the values for T_M given by Reber³:

$$T_s \text{ at } 36 \text{ GHz} = 186 \text{ K} \times 42.6 = 7,920 \pm 90 \text{ K};$$

$$T_s \text{ at } 37.75 \text{ GHz} = 185 \text{ K} \times 40.6 = 7,510 \pm 130 \text{ K}.$$

The absolute values of T_s are, of course, subject to the same uncertainties as the absolute value of T_M . The ratio of the two solar temperatures, however, does not have this uncertainty since T_M is nearly constant over the frequency range.

The slope, S , of the brightness curve between two frequencies f_1 and f_2 can be defined as:

$$S = \frac{\Delta T_s}{T_s} \cdot \frac{f}{\Delta f} = \left\{ 1 - \frac{(T_s/T_M)_{f_2} (T_M)_{f_2}}{(T_s/T_M)_{f_1} (T_M)_{f_1}} \right\} \frac{f_1}{(f_2 - f_1)} \quad (2)$$

where the (T_s/T_M) ratios are determined from equation (1). This definition of slope is the same as the geometric slope of a log-log curve and allows the ratios of the quantities to be used. Substituting the previous values of T_s/T_M for 36.0 GHz and 37.75 GHz into equation (2) gives

$$S = \left(1 - \frac{40.6}{42.6} \times \frac{185}{186} \right) \cdot \left(\frac{36}{37.75 - 36} \right) = 1.07 \pm 0.3$$

This result is shown in Fig. 1. Table 1 summarises the slope calculated here, the slope from the van de Hulst model and the slope from the Zheleznyakov model, and gives an estimate of the average slope of the observed disk temperatures from 20 GHz to 40 GHz obtained from Fig. 1.

Table 1 Values obtained for S , the slope of the brightness curve between f_1 and f_2

Observed points 20–40 GHz	$S = 1.4$
Our results	$S = 1.1 \pm 0.3$
Zheleznyakov model ⁵	$S = 1.4$
van de Hulst model ⁴	$S = 0.18$

The steep slope measured at 36 GHz (Table 1) indicates that the observed disk temperatures in the 20–40 GHz range are probably more reliable than the van de Hulst model⁴ calculations. In the centimetre region the observed disk temperatures again join the van de Hulst curve (Fig. 1). There may be a region around 15 GHz where the slope becomes zero, and one would expect no limb brightening to occur. A measurement of the slope between 40 GHz and 50 GHz would determine how the curve blends in with the nearly flat slope found by Reber³ at 50 GHz.

We suggest the future measurements of the solar brightness temperature at millimetre wavelengths should include a measurement of the new Moon. The new Moon serves as a stable calibration source outside the Earth's atmosphere, so that observations of the Sun made with different instruments at different frequencies can be directly compared by considering their Sun to new Moon ratios.

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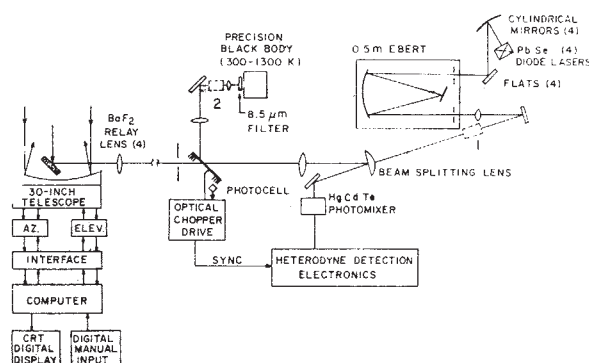
Infrared heterodyne spectroscopy of astronomical and laboratory sources at 8.5 μm

THE first successful infrared heterodyne spectrometer featuring semi-tuneable semiconductor diode lasers was constructed and used near 8.5 μm to make laboratory measurements of line profiles in N_2O and to detect thermal emission from Mars and from the Moon. This experiment was conducted at the coude focus of the 30-inch telescope at the Goddard Optical Research Facility in January and February 1974.

In heterodyne detection, infrared radiation (possibly from a remote source) is mixed with the output of an intense coherent local oscillator and a signal is detected at the difference frequency (called the intermediate frequency or i.f.). The spectral characteristics of the remote source are preserved at the i.f., except that the frequency scale is effectively translated by an amount equal to the local oscillator frequency. Using the very best available infrared detectors, and preamplifiers, the i.f. bandwidth can extend from close to 'direct current' to above 1 GHz and radio detection techniques may then be used to determine the fine structure of the spectrum of the remote source. The limiting spectral resolution is set by the spread in the local oscillator frequency which for semiconductor diode lasers can be less than 10^5 Hz (ref. 1). Thus, spectral resolutions exceeding $1:10^8$ are possible, far in excess of the resolutions attainable with conventional infrared spectroscopic techniques². Heterodyne spectroscopy is ideally suited for the identification of atomic and molecular species in remote infrared sources and for determination of the line profiles, giving information on the kinetic energy distributions, turbulence conditions, and Doppler velocities of the sources. The extremely high spectral and spatial resolutions make possible the study of low density, low kinetic temperature astronomical sources such as stars, comets, interstellar clouds and the upper atmospheres of planets.

Heterodyne radiometric (non-spectroscopic) methods using gas lasers as local oscillators have recently been successfully applied to detection of broad-band thermal radiation from astronomical sources³⁻⁷ and to laboratory detection of pollutant gases⁸. Although these results demonstrated the feasibility of making such measurements, the real power of heterodyne detection lies in remote spectroscopic measurements at high resolution, long wavelength, and high sensitivity. Peterson *et al.*^{9,10} made the first heterodyne spectroscopic measurements of C^{18}O_2 absorption lines in the Mars atmosphere near 11 μm (see also ref. 10). Their device featured a CO_2 local oscillator and so their observations were limited to the intermediate frequency bandwidth (~ 1 GHz) on either side of the discrete local oscillator frequencies. The versatility of a heterodyne spectrometer is limited by the frequency range over which the laser local oscillator may be tuned and still have sufficient power and coherence for heterodyne operation. Continuously tuneable

Fig. 1 Spectrometer (8.5 μm) optical system.



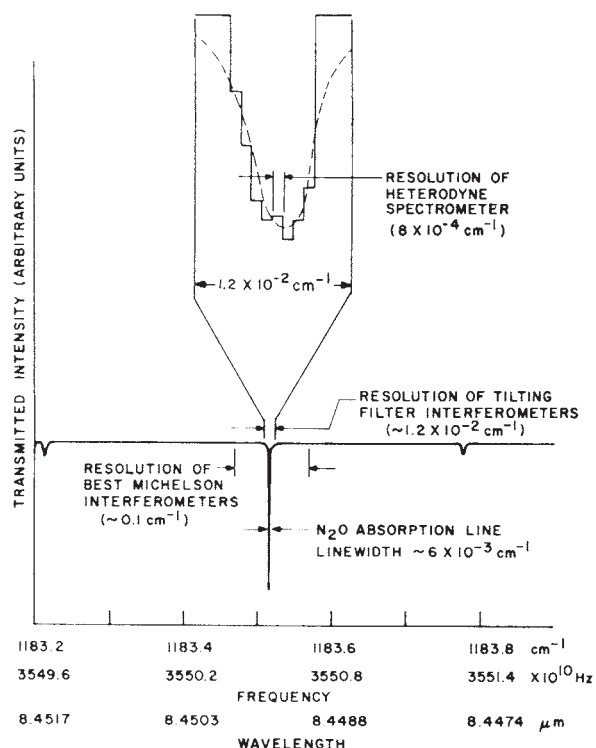


Fig. 2 Comparison of spectral resolution of the heterodyne with other high resolution techniques near 8.5 μm.

(over several hundred wave numbers) lasers are not yet commercially available in the middle infrared, but semi-tuneable lasers have recently become available.

Cryogenically cooled semi-tuneable semiconductor diode lasers^{1,11} have been proposed as possible local oscillators for remote heterodyne measurements of air pollutants by Hinkley and Kelley¹². Diode lasers emitting at nominal wavelengths from 5–34 μm can now be manufactured¹³ and are commercially available (from A. D. Little, Inc.). The nominal wavelength can be preselected by varying the chemical composition of the ternary semiconductor compounds. These lasers generally show multimode output, with mode separations of up to several wave numbers. Each mode can be current tuned continuously over ~1 cm⁻¹ (~3 × 10¹⁰ Hz at 8.5 μm). Thus, tuning is truly continuous over each single mode and piece-wise continuous over the wave length range of multi-mode operation.

We have built a heterodyne spectrometer using PbSe semiconductor diode lasers as local oscillators, a HgCdTe photodiode as a photomixer¹⁴, and an 8-channel filter bank as a line receiver. The spectrometer was interfaced with a computer controlled 30-inch telescope, and a Dicke type chopper was used to look alternately at the astronomical source and a precision blackbody reference source (Fig. 1). An array of four current-tuned diode lasers provided adequate local oscillator power for direct absorption measurements over nearly the entire 1,160 to 1,190 cm⁻¹ region; however, the range over which the power was sufficient for heterodyne detection was considerably smaller (~5 cm⁻¹). The maximum total power output from our best diode was ~2 mW of which ~100 μW of single mode coherent power was actually incident on our photomixer.

For heterodyne detection the laser and source signals were superimposed by the beam-splitting lens and focused on to the photomixer (Fig. 1). A 200-MHz band at the intermediate frequency output of the photodiode was then fed into the eight-channel filter bank and the output voltage from each 25 MHz channel was linearly converted to a frequency which was counted with a multichannel analyser for the period of integration. Data acquisition was synchronised with the chopping frequency. The measured i.f. noise-equivalent-power (NEP) for our system was 1.5 × 10⁻¹⁹ W Hz⁻¹ at 10.6 μm and 100 μW

local oscillator power. At 8.5 μm and 100 μW the extrapolated NEP is 3.6 × 10⁻¹⁹ W Hz⁻¹. This is a factor of two higher than the theoretical limit $h\nu/\eta$, where η = quantum efficiency) and about a factor of two below the result derived from blackbody measurements at 8.5 μm. Neglecting optical losses and taking into account the marginal laser power, the minimum detectable power for our system for 8-min integration times was ~1 × 10⁻¹⁶ W in a bandwidth of 25 MHz. The resolving power of our heterodyne spectrometer was 1.4 × 10⁶.

A comparison of the resolution of our heterodyne spectrometer with other high resolution spectroscopic techniques used to measure remote sources near 8.5 μm is given in Fig. 2. A portion of the N₂O spectrum near 8.5 μm is shown along with the resolving bandwidth of the Michelson, tilting filter and heterodyne techniques. Only the heterodyne technique is capable

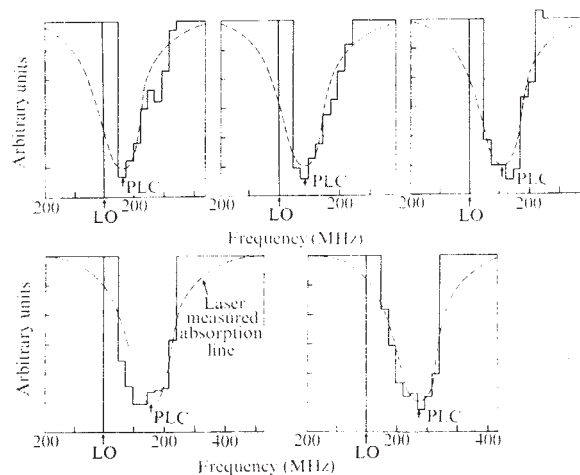


Fig. 3 Heterodyne detection of N₂O absorption line. ---, Absorption line profile measured in the direct detection mode. The histogram corresponds to the line profile measured in the heterodyne mode in the eight-channel filter bank.

of line profile measurements of the N₂O lines. Line profiles in the ν₁ band (100-000) of N₂O were measured directly and in the heterodyne mode (Fig. 3).

A cell filled with N₂O at 10 torr pressure was placed in position 1 (Fig. 1) and the absorption line positions and profiles were determined by direct detection of tuned laser line absorption. The cell was then placed in position 2 and the lines were heterodyne detected in absorption against a 1,300 K blackbody continuum (the optical path to the telescope was blocked). The line was then moved through our filter bank by tuning our local oscillator slightly between measurements. The pressure broadened absorption linewidth was about 170 MHz. For comparison purposes, both lines were normalised to the same amplitude and the direct detected line was drawn at the predicted line centre (PLC) position in the filter bank. The noise is greater in the heterodyne line profile because the blackbody power in a 25 MHz heterodyne channel corresponds to ~10⁻¹³ W (~10⁻¹⁴ W at line centre) whereas the direct measurements correspond to ~10⁻⁴ W of laser power at 8.5 μm. We believe that this is the first time that a molecular absorption line profile has been measured using both active and passive measurement techniques.

The heterodyne system was next applied to astronomical observations and was used to measure spectroscopically thermal radiation from Mars and from the Moon (Fig. 4). The blackbody continuum near 8.5 μm gave heterodyne signal-to-noise ratios of 3 to 8. The variations were caused by daily changes in the local oscillator power and changing atmospheric conditions. The theoretical system signal-to-noise ratio for a blackbody source is given by^{6,15} where B is the i.f. bandwidth,

$$S/N = \alpha \eta_{\text{eff}} (B \tau^{1/2} / (\exp(h\nu/kT) + 1)) \quad (1)$$

τ the integration time and α the transmission of the atmosphere and optics; η_{eff} is the effective quantum efficiency of our photodiode and is equal to the true quantum efficiency degraded by a factor stemming from the lack of sufficient local oscillator power

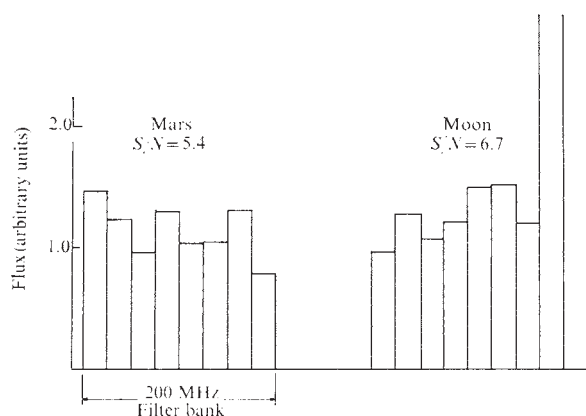


Fig. 4 Heterodyne signals at 8.5 μm from the Moon and Mars. The signal in each channel is represented by a bar whose height is proportional to the net flux received from the source. The huge signal in the last channel of the lunar data was probably a system noise pulse and was disregarded in determining the signal-to-noise ratio.

to reach the shot-noise limit. The true quantum efficiency of our photodiode at 8.5 μm was about 13% and η_{eff} was estimated to lie between 2% and 6.5% depending on laser operation.

Theoretical signal-to-noise ratios (with $\alpha = 1$ in equation 1) based on local lunar temperature at the region of observations, known electronic parameters, and the appropriate η_{eff} were from 5 to 10 times greater than the experimentally obtained values, implying $\alpha = 0.1 \rightarrow 0.2$. Optical transmission measurements gave $\alpha \approx 0.16$, which is consistent with the range of values obtained by comparing the experimental and theoretical S/N ratios discussed above.

The observed S/N ratios for Mars are more compatible with a temperature of 300 K than the expected value of $\sim 250^\circ\text{K}$ (refs 9, 10 and V. Kunde, private communication). The effective quantum efficiency changed between the measurements of Moon and Mars because of the greater laser output during the Mars run. The quantitative results are of only limited accuracy because of the frequent variation in laser power, poor telescope pointing stability, changes in zenith angle between runs and daily changes in atmospheric conditions. Accurate monitoring of all these parameters was not feasible at the time of the experiments.

The blackbody power from Mars incident on the heterodyne detector in a bandwidth of 25 MHz is $7 \times 10^{-16}\text{ W}$. Conventional techniques are more sensitive for broad-band measurements of blackbody radiation, but spatially localised, narrow spectral lines (with linewidths $\geq 100\text{ MHz}$, say) containing an equivalent energy would be undetectable using conventional techniques. The line profiles most certainly could not be observed. Although our astronomical measurements were not of spectral lines the power detected was of the order expected from remote line sources. The high resolution of our tuneable heterodyne spectrometer (as shown in the laboratory spectral line study) together with its high sensitivity (as demonstrated in the astronomical measurements) make it a suitable instrument for detecting and mapping narrow low intensity spectral lines over a broad wavelength range in the infrared.

Although we were able to obtain heterodyne signals with diode lasers as local oscillators, the measurements were far from straightforward. The coherent output power from presently available diode lasers is generally below that required for shot noise limited heterodyne operation. Because of the characteristics of the diode lasers, great difficulties were encountered in the design of the optical system to collect, focus and match the laser output to our heterodyne field of view. These problems can be eliminated by using better diode lasers and some success has already been achieved in growing more powerful lasers (see, for example, ref. 16). The output of the diode laser was sensitive to nearly all environmental effects such as room temperature variations, mechanical and acoustic vibrations, the liquid helium level and the heat sinking parameters of the helium Dewar. The high

resolution of our instrument requires stabilities of better than 0.6 p.p.m. in the focusing optics and the parameters of the diode laser. Meaningful measurements could only be made when the system remained stable during the period of data acquisition. Needless to say, conditions sometimes changed between measurements, making accurate comparisons between runs difficult.

These results demonstrate the capabilities of a heterodynespectroscopic system and that heterodyning can be achieved using semiconductor diode laser local oscillators. With improved design and better diode lasers future systems should approach the stage at which their operation is limited only by quantum noise.

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Origin of magnetite and pyrrhotite in carbonaceous chondrites

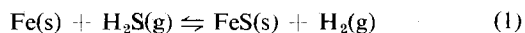
CARBONACEOUS chondrites, although comprising only about 2% of known meteorites, are extremely interesting for scientific investigation. Their mineral constitution, and the correspondence between their bulk chemical composition and the solar abundance of condensable elements, indicate that minimum chemical fractionation and thermal alteration have occurred. The mineral phases observed in these primitive chondrites are sufficiently unique, with respect to other meteorite classes, to have elicited considerable speculation about the physical environment in which they formed¹⁻⁷.

We consider here two minerals—magnetite and pyrrhotite—present in some carbonaceous chondrites and propose that a substantial fraction of the magnetite, at least, resulted from the

oxidation of troilite. Pyrrhotite is expected as a direct consequence of magnetite formation through this reaction. Previously, meteoritic magnetite was considered to have resulted from the oxidation of metallic iron⁸ or from the alteration of olivine⁹. No adequate genesis has been proposed for pyrrhotite¹⁰.

The formation of magnetite from troilite has been observed¹¹ during thermomagnetic analyses—measurement of saturation magnetisation against temperature—on meteoritic troilite. We observed similar behaviour during similar experiments¹² on carbonaceous chondrites and troilite separates from the Staunton iron meteorite, both under vacuum and in a fugacity-controlled atmosphere¹³. As magnetite was observed to form under vacuum, it is evident that troilite reacts with gaseous oxygen, rather than with the gases used for fugacity control. The fact that magnetite formed from troilite on a time scale of minutes prompted us to inquire whether meteoritic magnetite may have formed similarly.

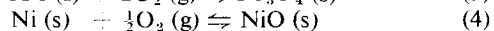
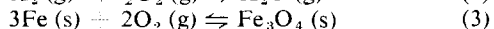
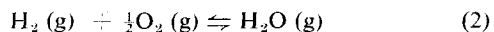
Urey⁸ concluded from thermodynamic arguments that, in an atmosphere with a solar composition of H_2 and H_2S , reaction (1) would proceed to the right below about 700 K. On that basis, meteoritic troilite is usually considered a stable phase below that temperature. The inferred 'stability', however, is relevant only with respect to H_2 and H_2S , to the exclusion of other gases and, therefore, yields no information on the reaction of troilite with O_2 .



Despite the ubiquitous presence of troilite in chondritic meteorites¹⁻⁷, few studies have been made of its stability and the possibility of its oxidation. Above 413 K, troilite converts to hexagonal low-temperature pyrrhotite¹⁴. Taylor¹⁵ has determined the approximate path that pyrrhotite (and presumably troilite) takes on oxidation in air. During oxidation, the sulphide mineral becomes increasingly iron deficient, with the eventual formation of the end member of the oxidation series, pyrite. Apparently, the effective fugacity of sulphur increases (without the addition of that element) whereas the iron activity decreases, the iron being partitioned into the oxide phase.

During thermomagnetic experiments on troilite (some conducted at constant temperature), we observed magnetite formation at temperatures as low as 373 K, provided that the oxygen fugacity was held in the magnetite stability field, and that the troilite was sufficiently finely divided. As expected for a grain-surface reaction, the rate and extent of reaction were strongly dependent on the grain size. Furthermore, we have observed thermomagnetically the formation of pyrrhotite, which confirms Taylor's work¹⁵.

If meteoritic magnetite resulted from the oxidation of troilite then the reaction must have been able to occur under conditions expected in the early Solar System. The petrological evidence must also be compatible with the expected consequences of magnetite formation by that means. Therefore, consider the oxygen fugacity equilibria for three reactions:



Since the oxygen fugacity in an atmosphere of solar composition is governed by reaction (2), the oxygen fugacity can be calculated as a function of temperature:

$$-\log_{10}(f_{O_2}) = (-2 \Delta G_f^\circ / 2.3RT) - 2 \log_{10}(H_2O/H_2) \quad (5)$$

where the ratio H_2O/H_2 is determined from cosmic abundances¹⁶ and ΔG_f° is taken from thermodynamic data. Oxygen fugacity equilibria for reactions (3) and (4) can also be calculated:

$$-\log_{10}(f_{O_2}) \rightleftharpoons -\Delta G_f^\circ / 2.3nRT \quad (6)$$

using the appropriate thermodynamic data and noting that for

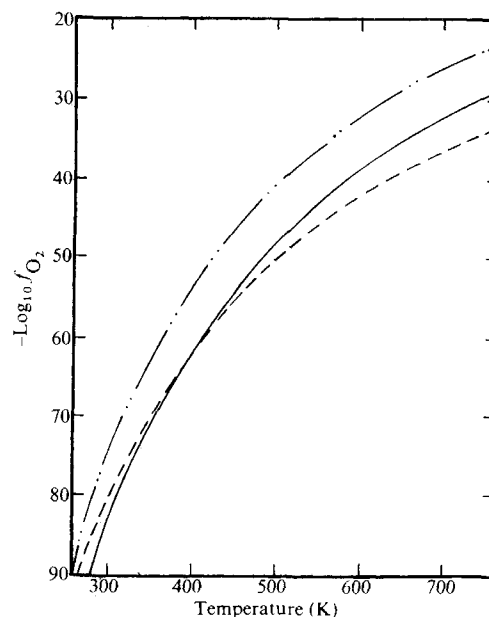


Fig. 1 Stability fields of Ni-NiO, Fe-Fe₃O₄ oxygen fugacity and the equilibrium oxygen fugacity expected in an atmosphere with a cosmic abundance of H_2 and H_2O . — · — · —, Equilibria of Ni-NiO oxygen fugacity. The NiO stability field lies above this curve, metallic Ni below. —, Equilibria of Fe-Fe₃O₄ oxygen fugacity. The magnetic stability field lies above this line, metallic iron below. — · — · —, Oxygen fugacity in an atmosphere with a solar abundance of H_2 and H_2O .

equations (3) and (4), $n = 2$ and $n = \frac{1}{2}$, respectively. For temperatures under consideration here, FeO equilibria can be ignored.

The results of these calculations are shown in Fig. 1. Below about 400 K the oxygen fugacity expected in an atmosphere with a solar composition of H_2 and H_2O lies within the magnetic stability field. These calculations demonstrate that provided that equilibrium was maintained, magnetite formation from condensed troilite is possible at temperatures at which we have determined that troilite can readily undergo oxidation. The oxygen fugacity expected in the early Solar System lies well within the metallic nickel stability field at all temperatures. Nickel oxide would not be expected, and any nickel present in the condensate must have existed in a metallic state or in some other chemical form, such as a sulphide.

Next consider the oxidation of meteoritic troilite under conditions expected in the early Solar System. As oxidation progresses, the sulphide mineral becomes iron deficient. Any nickel in the troilite should not oxidise at the oxygen fugacities expected, and, therefore, would remain in the sulphide phase. Thus, the sulphide mineral would become increasingly rich in nickel and increasingly deficient in iron. The degree to which sulphur is partitioned between the sulphide mineral and the gaseous sulphur phase may be a function of the prevailing sulphur fugacity in the system, and is difficult to assess. In a fully condensed system heated as high as about 400 K, the sulphur pressure will depend on whether the system is closed—as expected for subsurface regions of a planetoid—or open (even partially open)—as expected for small porous bodies, for near surface regions of the planetoid, or for material dispersed in space. In an incompletely condensed system, additional complications may arise as a result of sulphur present in the vapour phase. Nonetheless, it is possible to conceive of a fully condensed system subjected to mild heating and open to the loss of the gaseous sulphur phase. Such a system closely approximates the conditions existing during our experimental studies.

If meteoritic magnetite formed from troilite oxidation, as we

suggest, several consequences may be expected (and are observed) in meteorites. From the chemical arguments, discussed here, we expect that any magnetite formed from troilite would contain little nickel, even if the oxidation had been extensive: magnetite in carbonaceous chondrites has an extremely low Ni content^{13,17}.

We also expect the sulphide minerals resulting from troilite oxidation to attest to the degree of oxidation, thus, large grains, subjected to mild oxidation, should form pyrrhotite, somewhat enriched in Ni. Pyrrhotite has been observed in the Orgueil¹⁸ and Revelstoke¹⁹ meteorites, both of which contain abundant magnetite: 11.9% and 7.2% respectively¹³. The Ni content of the pyrrhotite phase of Orgueil (~1.0%) is significantly higher than that of meteoritic troilite (~0.2%). Unless an alternative mechanism is found, it seems that the observed pyrrhotite resulted from the oxidation of troilite, which verifies our thesis. Furthermore, extreme oxidation of troilite is expected to result in pyrite formation. The only meteorite in which pyrite has been observed²⁰ is the 'recrystallised' carbonaceous chondrite Karoonda: using thermomagnetic measurements we found that it also contains abundant magnetite (7.7%). Finally, the oxidation of troilite may yield a source of SO₂ for the formation of soluble sulphate species observed in carbonaceous chondrites¹. If so, the sulphur in the sulphate phase should be isotopically lighter than the sulphur remaining in the sulphide phase. For Orgueil, at least, that is the case²¹.

We propose that much (if not all) of the magnetite observed in carbonaceous chondrites resulted from troilite oxidation at temperatures $\lesssim 400$ K and that pyrrhotite formation is an expected consequence of this reaction. We have observed experimentally the oxidation of troilite over a time scale ranging from a few minutes to a few hours (depending on grain size and temperature) under conditions similar to those expected in the early Solar System. It seems that the previously inferred 'stability' of troilite in an atmosphere of solar composition, based on reaction (1), is invalidated by the very real possibility of reaction with oxygen. We suggest that further experimental work on the oxidation of troilite, specifically regarding the influence of sulphur fugacity, may yield data useful in establishing constraints on permissible modes of major element condensation and on metamorphic conditions present during the formation of chondritic meteorites.

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Detailed structure of a mantle seismic zone using the homogeneous station method

As the accuracy of location of mantle earthquakes has increased, the suggested thickness of seismic zones within the mantle has decreased¹ and special studies are now needed to determine the detailed structure of these zones. In this study we used the homogeneous station method to locate earthquakes occurring at intermediate depths beneath the North Island of New Zealand, and here report that the hypocentres could lie on two dipping surfaces about 9 km apart. Using the plate tectonics hypothesis these surfaces may be related to the top and bottom of a dipping, brittle zone, either down-going oceanic crust^{2,3}, or some colder region within the descending lithosphere⁴.

The intermediate zone under North Island strikes approximately north-west, dips at about 67° to the horizontal and is overlain by the stations of the seismic network run by the Seismological Observatory at Wellington. From the Observatory's readings for the period November 1965–December 1972 (excepting 1970, when readings were unavailable), all subcrustal earthquakes were selected which had iP or P-wave phases recorded at seven selected seismic stations (see Fig. 1) and had all but one of these phases recorded to the nearest tenth of a second; the others were recorded to the nearest second. The 73 earthquakes selected, which included 26 with one reading to the nearest second, were relocated using the standard least squares method, the seven P-wave readings, and the Jeffreys–Bullen tables.

The homogeneous station method, which requires that each station records the P-wave phase of each earthquake, is the simplest of the more accurate relative hypocentre location procedures (paper in preparation). The relative mislocation of a pair of events will depend only on the reading errors and the change, from one event to the other, in the model travel time errors—these are the discrepancies between the actual travel times from the true hypocentres to the stations and the times given by the travel-time tables used. Confidence ellipsoids for the relative locations were calculated using time residuals. Fifty-four of the hypocentres within, or close to, the edges of the station network were divided into eight geographical groups, the hypocentres within each group being within a radius of 40 km. If r_{ij} is the time residual of the j th station from the i th event in a group, then we have a model

$$r_{ij} = S_j + \varepsilon_{ij}$$

where the S_j s are the mean station residuals for the group and are related to the mean model travel time errors; and the ε_{ij} s are related to the reading errors and the variation of the model travel-time errors about their means. We assume the quantities ε_{ij} have independent normal distributions with zero mean and a variance σ^2 . The station terms S_j for each group were determined (see Table 1) and a pooled estimate of σ^2 was made for all groups. This gave an estimate of 0.39 s for σ , based on 114 degrees of freedom (54 · 7 residuals — 54 · 4 hypocentral parameters — 8 × 6 station terms). Thus, the 90% confidence ellipses are given by

$$(x, y) A(x) = 2(0.39)^2 F(2, 114) \quad (1)$$

where A is the appropriate 2×2 submatrix of the inverse of the condition matrix of the least squares equations and $F(2, 114)$ is the 90% value of the F distribution.

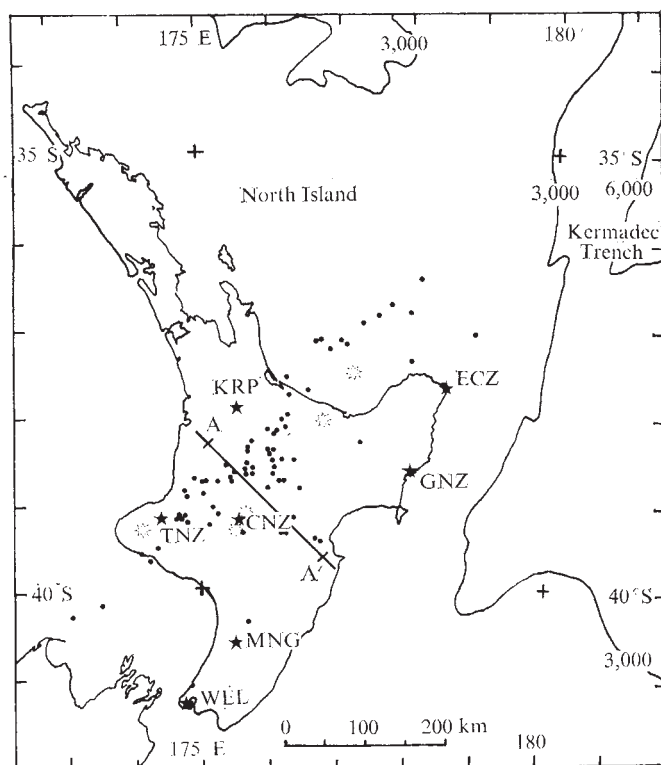


Fig. 1 Map and bathymetry of North Island. ●, Epicentres of the earthquakes considered; solid stars, seismic stations used; open stars, active andesite volcanoes*. Solid line, depth contours showing depth in metres.

A plane with strike N 45.25° E and dip 67.25° was fitted by the least squares method to all but one of the hypocentres which fall within the net and which were deeper than 118 km. The sum of squares of the perpendicular distances of these 49 hypocentres from the plane was minimised and the standard deviation of the distances was 5.6 km. The Seismological Observatory's hypocentres for the same earthquakes were distributed about a similar plane with a standard deviation of 10.0 km. Hamilton and Gale⁵ fitted median curves to Observatory hypocentres in the same area, and found standard deviations of about 13 km. Clearly, the homogeneous station method locates the hypocentres much more tightly. Typical 90% confidence ellipses in a vertical plane perpendicular to

Table 1 Residuals, stations means, sums of squares about these means, and hypocentre standard errors for one of the geographical groups of earthquakes considered in the text

	CNZ*	ECZ	GNZ	KRP	MNG	TNZ	WEL	Standard error
Residuals								
	0.3	0.5	-0.1	-1.1	-0.2	1.2	-0.7	1.3
	0.3	0.5	-0.4	-0.7	0.2	0.7	-0.6	1.0
	0.1	0.5	-0.1	-0.9	0.1	1.2	-0.9	1.3
	-0.1	0.2	0.1	-0.7	0.2	1.1	-0.8	1.2
	0.1	0.5	0.0	-1.0	0.0	1.3	-0.9	1.4
	0.4	0.2	0.4	-0.3	0.3	0.7	-0.6	0.8
	-0.3	-0.1	0.4	-0.6	0.2	1.1	-0.8	1.1
	0.0	0.2	0.3	-1.0	-0.3	1.4	-0.7	1.4
Mean	0.00	0.26	0.08	0.79	0.06	1.09	-0.75	
Sum of squares	0.46	0.59	0.55	0.46	0.32	0.43	0.10	

*For location of seismic stations see Fig. 1.

our plane, and given by equation (1), are shown in Fig. 2. The average standard deviation in the direction perpendicular to the plane, based on the estimate of 0.39 for σ , is 3.5 km. Thus, we have good accuracy perpendicular to the zone below about 120 km but not above that depth, where the zone becomes more horizontal. (The earthquake below 118 km excluded from the analysis is isolated and its epicentre is about 38.3° S, 117.2° E. Various depth determinations in kilometres are: ours 160, the Seismological Observatory's 189, US Coast and Geodetic Survey 114 and International Seismological Centre 100.)

Using the plane of best fit as a reference plane a quadratic surface was fitted to the 49 hypocentres by minimising the sums of the squares of distances from the hypocentres to the surface in the direction perpendicular to the plane. The standard deviation, 4.2 km, was significantly better than that for the plane, but more importantly it was noticed that although most of the hypocentres lay close to the surface, 10 widely spread

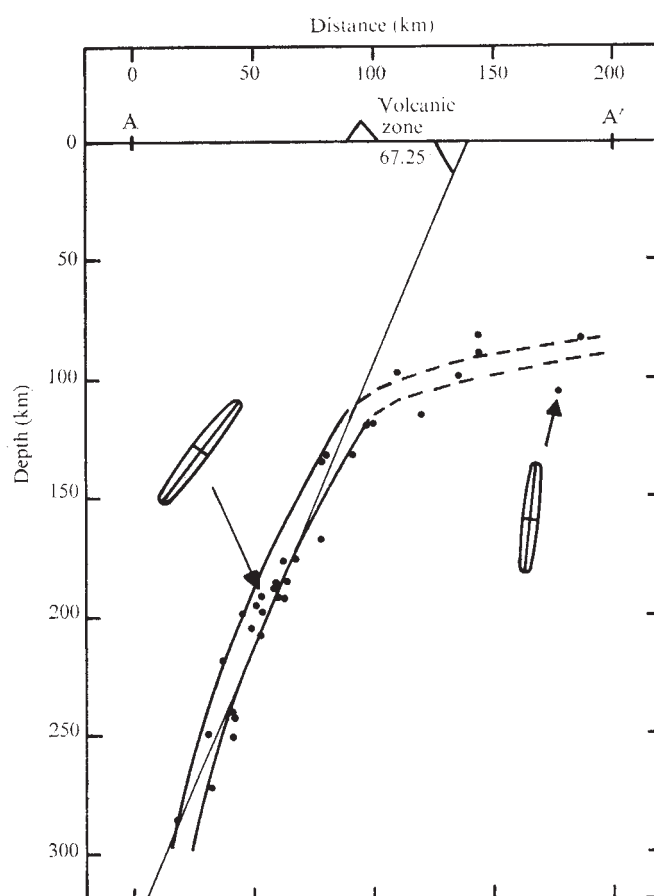


Fig. 2 Cross section of the seismic zone through AA' (Fig. 1), showing hypocentres (●) within 50 km of the section, typical 90% confidence ellipses of relative locations and sections through the best fit plane. —, The quadratic surfaces 9 km apart (described in the text); ---, possible extensions of these surfaces.

hypocentres were significantly above it in a direction perpendicular to the plane. A quadratic surface was fitted to the larger group of 39 earthquakes and the standard deviation of hypocentres about this surface was 3.4 km—remarkably close to the value of 3.5 km obtained independently from the residual analysis. The remaining 10 hypocentres had a mean displacement of 8.8 km from the surface (perpendicular to the plane). The standard deviation about their mean was 1.5 km, but this low figure probably results from the biased sampling technique. The histogram of distances from the surface is shown in

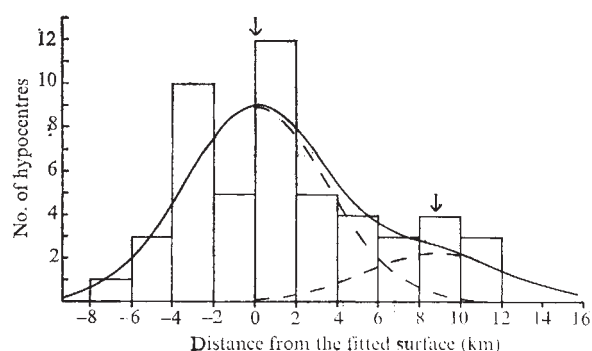


Fig. 3 Histogram of distances of hypocentres from the lower quadratic surface (see text), and the predicted distribution of 39 hypocentres on the surface of the plane and 10 hypocentres 8.8 km above the surface of the plane, with normal distributions and standard deviations about their means of 3.5 km.

Fig. 3 together with the predicted distribution for 39 hypocentres on the surface of the plane, and 10 hypocentres 8.8 km above the surface of the plane, with normal distributions and standard deviations of 3.5 km about the means in both cases.

The evidence suggests strongly that the North Island deep seismic zone is about 9 km thick and that the earthquakes initiate in the top and bottom surfaces of the zone. Mechanism studies⁶ show no clear differences between earthquakes originating on either surface, and our results differ, therefore, from those from the Kurile Islands⁷ where there is a 30 km thick zone with differing earthquake mechanisms above and below. Further studies are to be done to locate less well recorded earthquakes and to improve the absolute locations.

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Possible large creep event apparently preceded by a dilatant precursor

THE invar wire earth strainmeter at the Nelson underground field station of the Physics and Engineering Laboratory, New Zealand, has produced the record shown in Fig. 1. The size of the two strain steps shown was far too great to be recorded on the tidal chart recorder but have been established with complete confidence from the amount of mechanical adjustment needed at the time of the inspection visits. Long term invar expansion has been removed from the curve shown. This is measured independently on an identical instrument decoupled from the earth using a sample of the same wire held at the same temperature and tension, but referred to very old large diameter Pyrex glass tubes as a reference length. Ideally, these should be of fused silica. The equation of the long term expansion agrees quite closely with that reported by Kaye¹ in 1911.

There were no earthquakes at the times of the two events shown and it is postulated that the strain change of 1.2×10^{-5} was produced by a large creep of about 20 cm, possibly on the nearby Waimea fault. On the surface this lies 5 km from the station but the creep was perhaps deep and without surface expression. In contrast to California, most seismic events in New Zealand do not correlate well with known active faults², and this event may be no exception. The apparent precursor about 20 months before would, if caused by rock dilatancy, indicate an earthquake of about magnitude 6 (ref. 3), but instead an aseismic creep event occurred. Work by Chinnery⁴ indicates that a 20 cm displacement is of the order to be expected from an earthquake of magnitude 6. The second much smaller event is regarded as an 'after-creep' of the first.

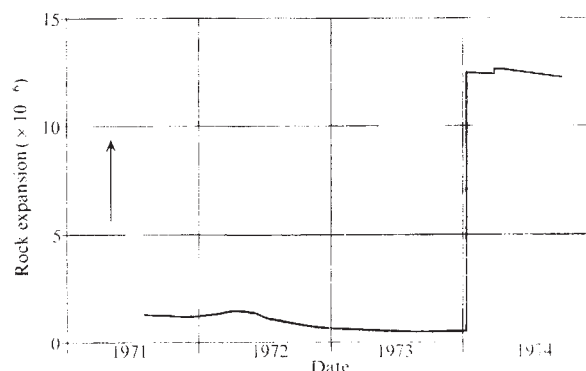


Fig. 1 Long term earth strain record from Nelson underground field station (41.5° S, 172.9° E; 150 m deep) from the time recording began in July 1971. The installation of the instrument⁶ was carried out in early February 1971. The curve has been derived both from measurements at the time of the inspection visits and from the high sensitivity chart recorder which has an automatic stepper giving an effective range of approximately 70 chart widths.

Wire tension increased at the time of each event and therefore they can hardly be attributed to instrumental defects. No other comparable events have been recorded on two other strainmeters of the same design which have been operating at other distant stations for similar periods of time. There is also a difference in the rate of strain accumulation before and after the event. Extrapolation of the present rate would indicate another such event in approximately 20-25 yr which, of course, might then appear as an earthquake. It is interesting that in 1941 there was an earthquake of magnitude 5.5 at just this location⁵ (41.6° S, 172.9° E), although at a depth of 80 km.

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Mechanism for the persistence of tectonic lineaments

It is widely recognised that major faults and other linear zones of tectonic activity may remain intermittently active through extended periods of geological time: I suggest a mechanism which may account for this persistence.

Examples of long lived linear tectonic belts are known in most continents although their significance was probably

first appreciated in the Soviet Union where activity on the deep faults bordering the southern margin of the Siberian shield is known to extend from Archaean to late Phanerozoic times¹⁻³. In Africa the present rift zone has been identified as the locus of Precambrian activity⁴, and Precambrian lineaments in the West African and Guyana shields were reactivated during the Mesozoic separation of Africa and South America⁵.

Two of the major Precambrian boundaries in Greenland have been shown to have remained active for up to 1,000 Myr from their initiation in the Archaean⁶, and more recent work on the Nagssugtoqidian boundary has extended the probable period of intermittent activity to 2,500 Myr. The earliest recognised activity at this boundary is large scale ductile transcurrent movement along⁷ a vertical shear belt, 35 to 40 km wide, along which movement began about 2,650 Myr ago. Following further activity in the Precambrian, the boundary region was the locus of intrusion of a suite of kimberlite and associated dykes of Cambrian age⁷. The suggestion that movements continued during the Phanerozoic⁸, based on the possibility of pseudotachylites cutting dykes of the kimberlite suite, has since been confirmed (J. Grocott, personal communication). Offshore seismic work in connection with hydrocarbon prospecting has shown faulting or fault controlled deposition of Mesozoic sediments along the line of the Precambrian shear zone. This not only confirms that the lineament has remained intermittently active for more than half of the Earth's history, but also illustrates the economic significance of such lineaments.

Any explanation for the persistence of activity along a major linear movement zone is subject to two principal constraints. The persistence of activity must be provoked within the lithosphere because, over the time scale considered, no part of the lithosphere is likely to have remained fixed in position relative to underlying mantle. On the other hand, translation of plates and displacements within them result from causes external to the lithosphere. Both these requirements are met if a lineament is the expression of a persisting mechanical weakness, along which displacements which otherwise would be more widely distributed have been localised. If that is the case a lineament is likely to accommodate a variety of movement directions during its active life. An appropriate mechanical weakness would be one affecting a major part of the thickness of a lithospheric plate and would not be restricted to the relatively thin upper portion where brittle fracture is dominant in rock failure and displacement.

The significance of large scale zones of ductile simple shear strain has become apparent recently^{9,10}. In particular, it is clear that they are the deeper and mostly aseismic continuations of structures which appear at higher crustal levels as major faults, thrusts, and brittle fracture zones. A characteristic of ductile shear zones, most easily seen in small scale examples up to a few metres wide, is that the deformation, although plastic, is usually accompanied by a reduction in grain size. Theoretical and experimental studies^{11,12} suggest that grain size is a significant variable in determining rates of plastic deformation, because of the predominant role of grain boundary diffusion in deformation at high temperature and low stress. A theoretical model in which strain rate varies in inverse proportion to grain diameter¹³ is in substantial agreement with strain rate experiments on the deformation of marble at high temperature¹⁴: other theoretical studies indicate the importance of grain size in determining creep rates¹⁵. An acceleration of strain rates is to be expected towards the centre of ductile shear zones where, because of boundary effects, initial strains are highest and the initial reduction in grain size most marked. In a rock mass which is anisotropic in respect of grain size, and hence ductility, the application of deviatoric stresses will produce strain along suitably oriented

existing planes of finer grained and more ductile rock—those zones along which previous ductile displacement took place without subsequent recovery of grain size by grain growth. It is then important to determine whether or not planar zones of finer grained rock, seen at present levels of erosion in Precambrian basement rocks as ductile shear zones, extend through an appreciable thickness of the lithospheric plate. The scale of transcurrent displacements along major lineaments, whether now observed as brittle faults or ductile shears, is commonly of the order of tens or hundreds of kilometres and difficult to account for otherwise than by extension of the displacement downwards as far as the decoupled zone at the base of the lithosphere. The identification of intense tectonic fabrics in upper mantle rocks now seen as inclusions in kimberlites, dykes and pipes is particularly interesting: these fabrics have been particularly well demonstrated in a series of deformed mantle rocks from kimberlites in Lesotho¹⁶. Comparable fabrics exist, although less well developed, in the kimberlites from the Nagssugtoqidian boundary. Such examples, with textures identical with those of deformed crustal rocks, show the similarity of deformation processes at widely separated levels within the lithosphere, and demonstrate that ductility increase by grain size reduction can reasonably be regarded as likely to be effective on the scale of the lithospheric plate. Such weakening can only be responsible for the persistence of lineaments if recovery of grain size by grain growth is not effected in the time interval between successive increments of movement along the lineament. Recovery times are certainly very prolonged in crustal rocks as evidenced by the ubiquity of grain size anisotropy in tectonites, and by the absence of grain growth fabrics in the inclusions from both Lesotho and Greenland kimberlites, which suggests that the rates of recovery of grain size in mantle rocks are also slow relative to strain rates.

So I suggest that the concept of mechanical weakening through reduction in grain size may account for the longevity of major tectonic lineaments; this proposal can be tested by work on the fabrics of crustal and mantle tectonites, and on annealing rates. Future work should also show whether the distribution of tectonised mantle inclusions in kimberlites is related to tectonic lineaments, or whether kimberlite distributions are related to such lineaments as seems to be the case on the Nagssugtoqidian boundary and has been suggested for kimberlites in Southern Africa¹⁷ and Siberia¹⁸.

I emphasise that these proposals are complementary to current plate tectonic theory; plate boundaries initiated in recent geological time (the late Phanerozoic), in several cases seem to coincide with older lineaments.

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The February–June weather relationship in north-west Europe

It has been well said by Bonacina¹ that everything which happens to, or in, the atmosphere, affects its subsequent behaviour. In other words, there is a “memory” in the atmosphere, probably such that an anomaly produced at one time may lead to a similar, or a related, anomaly being restored in the future. It is known that although some factors can be neglected for short range forecasting, they become progressively more important in the longer range. These factors are unknown, although most meteorologists would probably agree with Namias² that two of the most important must be extraterrestrial events, such as variations in solar activity, and variations in the character of the Earth’s surface.

But empirical methods of long range forecasting are sometimes found simply by discovering relationships between observations at different times and places, and there seems no reason for not using them in prediction. The physical mechanism behind the relationship may not be fully understood, but the empirical discovery usually points to the research required for understanding the mechanism. Here I illustrate this idea by an example.

In the course of looking at climatic trends in north-west Europe, particularly the northern North Sea area, certain relationships were noted between weekly or monthly periods in the winter season and periods of similar length in the following summer. This may best be illustrated by what one may call the February–June relationship. Figure 1 shows the

Fig. 1 February and June mean temperatures, °C, Dalen i Telemark, 1940 onwards. (Pre-1950 shown by crosses.)

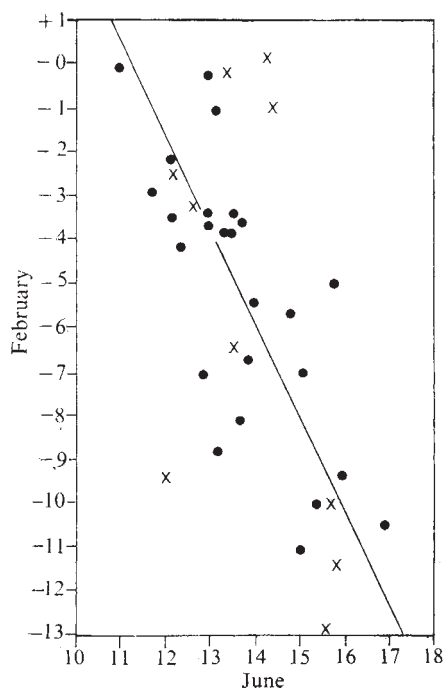


Table 1 Dalen i Telemark, mean temperature in June (°C)

	Forecast	Actual	Trend from previous June correct (C)	Forecast within 1° C of actual (C)
1939	—	13.6	—	—
1940	16.5	15.8	C	C
1941	15.9	15.7	C*	C
1942	15.6	12.0	C	
1943	11.3	13.4		
1944	12.4	12.2	C	C
1945	12.9	13.2	C	C
1946	12.8	12.7	C	C
1947	17.3	15.5	C	
1948	14.2	13.5	C	C
1949	11.1	14.3		
1950	13.0	13.0	C	C
1951	13.2	12.9	C*	C
1952	13.0	12.6	C*	C
1953	13.8	15.9	C	
1954	14.3	13.3	C	C
1955	14.4	12.9		
1956	15.0	14.3	C	C
1957	13.3	13.4	C	C
1958	14.8	13.7	C	
1959	13.8	14.1	C	C
1960	14.4	15.1	C	C
1961	13.3	13.8	C	C
1962	12.8	12.1	C	C
1963	15.0	15.4	C	C
1964	13.2	12.2	C	C
1965	12.9	13.3	C	C
1966	15.6	15.1	C	C
1967	13.2	13.6	C	C
1968	13.9	14.9	C	C
1969	15.1	16.0	C	C
1970	15.5	16.8		
1971	12.5	13.2	C	C
1972	13.4	12.4		C
1973	12.5	14.4	C	
1974	12.6	13.9	C	

* Almost the same as previous year.

relationship between February and June from 1940 onwards for Dalen i Telemark (59°27'N, 8°0'E), an inland station in southern Norway. A regression line can be drawn which shows that the mean temperature in February is inversely related to the mean temperature in June. If this regression line is used at the end of February to estimate the mean temperature for the following June, the results compare very favourably with the actual temperatures (Table 1).

It will be seen that in 30 years out of 35 the estimate was correct in the sense that it predicted correctly whether the June mean temperature would be higher or lower than (or very nearly the same as) that of the previous year. In 25 years out of 35 the mean temperature was predicted correctly to within 1.0° C and in 14 of those years it was correct to within 0.5° C. The accuracy of the estimates increased until the past few years, when it has deteriorated slightly. Some variations in this simple predictive method were investigated, mainly to try to take into account year-to-year trends, but this has not led to any general improvement in the method.

Periods other than calendar months were not used because of the greater amount of work involved. Had this been done, it would have rectified some, if not all, of the least successful predictions. For example, the prediction of a very warm June in 1955 proved to be incorrect, but in fact July proved to be exceptionally hot.

This February–June relationship was explored (1) backwards in time, and (2) in neighbouring areas. At Dalen i Telemark, during most of the 1940s, the relationship came close to the regression, but this was not the case from 1891 to the 1940s. There was, however, a suggestion of two populations, one with a direct correlation and another with an inverse correlation, the latter applying to all cases where the February mean temperature was -6.0°C or below. Examination of other stations in Norway, Denmark, Scotland and England indicated that the inverse correlation between February and

June mean temperatures had been spreading south and west. There is more scatter about the regression line at coastal stations, but, for practical purposes this is compensated for by the angle of slope of the regression line approaching 45°.

In both Norway and Scotland, higher than average temperatures in winter correlate well with higher than average rainfall, since both occur in westerly and cyclonic conditions. Conversely, there is relatively little precipitation in anticyclonic conditions. In summer there is an inverse relationship between temperature and rainfall. Thus forecasting temperature is almost tantamount to forecasting approximate rainfall. In the case of Bergen, Norway, in 22 consecutive years out of 23, June rainfalls were correctly predicted at least to the extent of being greater or less than in the preceding year, and in 13 years out of 25 the amount was predicted to within 25%. (Table 2).

Mean February temperatures from Dalen i Telemark were used to predict mean June temperatures and rainfall from 1954 onwards for Kinlochewe, in Wester Ross. For temperature, the predictions were nearly as good as when February temperatures for Kinlochewe were used, but for rainfall they were usually better (Table 3). The particular case of 1974 is interesting. February was mild, and followed a quite exceptionally mild January. The predicted rainfall for June was 165 mm. In the event, the June rainfall was only 69.6 mm at Kinlochewe, but there was a very marked spell of wet weather, preceded and followed by dry conditions, commencing on May 18; the rainfall for this period was 164.6 mm, suggesting that the very mild and wet period in January–February was preconditioning a wet spell in May–June.

The meaning and physical explanation of this empirical prediction technique can only be guessed at. The second of Namias' factors—variations in the character of the land or sea surface—may be a more direct key to the relationships described than is the extraterrestrial factor. Can one determine, for instance, what was the particular state of which part of the Earth's surface in successive Februaries which injected a factor into the atmospheric circulation that was effective in inducing another particular condition the following June? It is not unreasonable to suppose that the surface conditions in February reflect the dominating influence which has characterised the whole winter.

Two significant factors which come to mind are (1) the generally accepted spread southwards of the Arctic high pressure

Table 3 Kinlochewe, June rainfall (mm)

	Forecast		Actual	Trend correct compared with previous year (C)		Forecast correct within 50 mm (C), within 25 mm (C*)	
	X	Y		X	Y	X	Y
1954	110	112	123	—	—	C*	C*
1955	125	102	82		C	C	C*
1956	120	75	75		C	C	C*
1957	140	145	82	C	C		
1958	135	82	60		C		C*
1959	170	125	201	C	C	C	
1960	110	170	190	C	C		C*
1961	175	147	137	C	C	C	C*
1962	165	170	190	C	C	C*	C*
1963	105	70	76	C	C	C	C*
1964	165	150	103	C	C		C
1965	145	165	164	C	C	C*	C*
1966	135	45	149	C	C	C*	
1967	175	147	124		C		C*
1968	110	120	120	C	C	C*	C*
1969	105	70	45	C	C		C*
1970	110	50	66	C	C	C	C*
1971	170	182	94	C	C		
1972	140	140	150	C	C	C*	C*
1973	140	182	177		C	C	C*
1974	165	180	70	C			

X—using Kinlochewe February mean temperature.

Y—using Dalen i Telemark February mean temperature.

area, and (2) the related decrease in westerly days in the zone to the south of this. It is significant that Trondheim, the northernmost station considered, showed the inverse February–June correlation earliest, and that it became recognisable further south and west as time went on. This implies increasing anticyclonic control of the relationship. Markedly anticyclonic and cold Februaries in Norway have almost invariably preceded warm Junes, and since well before the 1940s; but in addition it seems that milder, westerly Februaries now precede cool wet Junes more regularly than they used to.

In view of the very limited success of the methods at present used by the Meteorological Office in producing monthly weather outlooks³ I feel that studies of the type described here could be very useful ingredients in the development of long-range forecasting technique, especially when correlated with the *Grosswetter Lager* and other atmospheric circulation studies. I am pursuing this matter, with some success, in cases other than the February–June relationship.

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Table 2 Bergen (Frederiksberg), rainfall in June (mm)

	Forecast	Actual	Trend correct compared with previous year (C)	Forecast correct within 50 mm (C)
1950	160	203	—	C
1951	163	72	C	
1952	140	225	C	
1953	132	46	C	
1954	115	137	C	C
1955	95	57	C	C
1956	90	165	C	
1957	150	112	C	C
1958	100	26	C	
1959	155	183	C	C
1960	135	166	C	C
1961	180	223	C	C
1962	155	116	C	C
1963	80	68	C	C
1964	153	252	C	
1965	147	187	C	C
1966	105	106	C	C
1967	177	163	C	C
1968	125	132	C	C
1969	80	80	C	C
1970	100	78	C*	C
1971	175	69		
1972	153	247	C	
1973	157	130	C	C
1974	195	131	C	

* Actual precipitation almost the same as previous year.

Molecular complexity of water vapour and the speed of sound

EVIDENCE has been given for a high binding energy of dimerisation in water vapour deduced from measurements of the speed of sound¹. The quoted value of 0.5 eV per molecule must be reconciled with values of about 0.2 eV per molecule which have been derived both from thermodynamic measurements² and from molecular orbital calculations³. We are interested in the behaviour of tropospheric water vapour, so we made observations at lower temperatures than the *PVT* and calorimetric observations which were relevant to steam technology. We

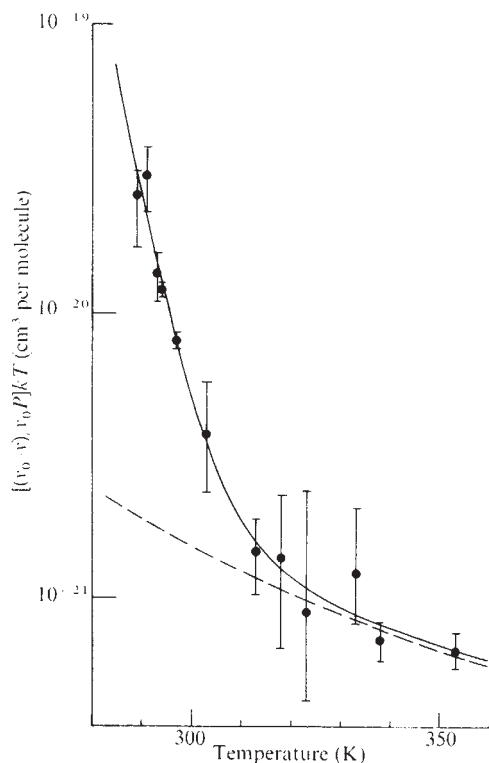


Fig. 1 $[(v_0 - v)/v_0 P]kT$ plotted against temperature. A two-dimer model fitted to the observations is represented by the solid line; for comparison, the dotted line is derived from published calorimetric measurements made in the temperature range 312–398 K. The points with error bars are derived from speed of sound measurements.

have now extended the temperature range of the speed of sound observations to cover the interval from 289 to 353 K.

Figure 1 shows values of the quantity $[(v_0 - v)/v_0 P]kT$ plotted against temperature T , where k is Boltzmann's constant. At each temperature the value of $(v_0 - v)/P$ was determined from the slope, and that of v_0 from the intercept of the straight line representing the observed speed of sound, v , against pressure, P .

Virial coefficients are used as the basis of the comparison of results, and are related to speed of sound by the expression⁴:

$$\frac{(v_0 - v)}{v_0 P} kT = - \left[B + B'T(\gamma_0 - 1) + B''T^2 \frac{(\gamma_0 - 1)^2}{2\gamma_0} \right] \quad (1)$$

where B is the second virial coefficient, the primes denote differentiation with respect to temperature, and γ_0 is the ratio of specific heats for water at the limit of zero pressure. We take γ_0 to have the value 1.32. Only binary interactions have been considered, as within the limits of experimental error we have observed that changes in the speed of sound at a given temperature are linearly proportional to changes in pressure.

Published values of virial coefficients for water have been reviewed critically by Wexler and Greenspan⁵ and we have chosen for comparison those derived by Goff and Gratch⁶ from calorimetric measurements^{7,8} made in the temperature range

from 312 to 398 K. The dotted curve in Fig. 1 is a plot of the function given by the right hand side of equation (1) using the empirical formula for the second virial coefficient, found by Goff and Gratch to give the best fit to the calorimetric data. At temperatures where our observations overlap with the calorimetric data they lie close to the line but at lower temperatures they depart considerably from it.

The second virial coefficient, B , has been represented² as the sum of a dimerisation term, B_2 and a term, B_1 , attributed to non-polar van der Waals' type attractions. Berthelot's formula allows B_1 to be calculated and for that formula we take the values of the critical temperature and pressure given by Rowlinson². For B_2 we use the expression:

$$B_2 = - \frac{N_2}{N_1^2} = - \sigma \exp\left(-\frac{E}{kT}\right) \quad (2)$$

where N_1 is the concentration of monomers, N_2 of dimers, and $-E$ is the dimer binding energy. σ is a scale factor for dimer concentration, related to the partition functions of monomer and dimer, and is taken to have negligible variation with temperature. The Rowlinson formulation of the second virial coefficient with the values $-E = 0.17$ eV per molecule, and $\sigma = 1.4 \times 10^{-24}$ cm³ per molecule gives a good fit to the calorimetric data.

To account for the speed of sound at low temperatures we have used a model for water vapour in which B_2 is itself the sum of two terms B_{2a} and B_{2b} , each applying to a species of dimer with a characteristic binding energy and scale factor. The full line in Fig. 1 represents a calculation using equation (1) and $B = B_1 + B_{2a} + B_{2b}$, with:

$$\begin{aligned} -E_a &= 0.16 \pm 0.09 \text{ eV per molecule } (\sigma_a \sim 10^{-24} \text{ cm}^3 \text{ per molecule}) \\ -E_b &= 1.4 \pm 0.3 \text{ eV per molecule } (\sigma_b \sim 10^{-45} \text{ cm}^3 \text{ per molecule}) \end{aligned}$$

These were found by least squares analysis to give the best fit of the curve to our observations. Representative values of the second virial coefficient and its components are given in Table 1. In the temperature range we have studied, the values of B do not depart by more than the experimental uncertainty from values calculated from the empirical formula of Goff and Gratch⁶. At low temperatures, the significant difference between the two curves in Fig. 1 may be attributed instead to large values of $-B_{2b}'$.

The binding energy of 0.5 eV per molecule given in the preliminary analysis in which only a single species was considered, is now explicable as a weighted average of the binding energies of two species of dimer. In our new analysis, values for the binding energy and the corresponding scale factor for one species are substantially in agreement with those of the familiar low binding energy dimer cited in the introduction. Comparing values of B_{2b} with those of B_{2a} we find that at 289 K the concentration of the high binding energy dimer is about 20% that of the low binding energy species. This indication of an appreciable concentration of dimers with a high binding energy is a provocative result which will need further investigation. We note that it applies to adiabatic conditions given by sound pulses which in our experiment had a duration of about 20 μ s.

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Table 1 Second virial coefficients (cm³ per molecule)

	289 K	312 K	353 K
B_1 (Berthelot)	-8.3×10^{-22}	-7.0×10^{-22}	-5.4×10^{-22}
B_{2a}	-1.4×10^{-21}	-8.8×10^{-22}	-4.5×10^{-22}
B_{2b}	-2.8×10^{-22}	-5.1×10^{-24}	-1.4×10^{-26}
$B = B_1 + B_{2a} + B_{2b}$	-2.5×10^{-21}	-1.6×10^{-21}	-9.9×10^{-22}
(present work)			
B (ref. 6)	-2.3×10^{-21}	-1.6×10^{-21}	-9.3×10^{-22}

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Fusion ignition of microfission explosions

WINTERBERG¹ proposed that microexplosions could be caused in very small amounts of fissionable materials such as ²³³U, ²³⁵U, and ²³⁹Pu (see also refs 2 and 3). A small critical mass could be created by imploding the fissionable material until it was 200–300 times its normal density. This could be achieved using a high energy, properly shaped laser pulse (of about 1 ns duration). For a chain reaction to begin in the assembled critical mass, however, neutrons must be present to initiate the fission process. For a critical mass of uranium, of 0.34 g, and a hydrodynamic disassembly time of 10^{-9} s, as proposed by Winterberg¹, conventional neutron sources will not be adequate to initiate a chain reaction. Initiation of the fission process using neutrons produced from the fusion of a deuterium–tritium (D–T) mixture placed in the core of the fissionable material seems feasible and offers several distinct advantages over conventional neutron sources.

As far as conventional neutron sources are concerned, a beryllium- α source of sufficient strength to initiate a chain reaction within of the order of 1 ns would be too large to fit within the uranium sphere (it would have an initial radius of 0.16 cm). For example, Hanson⁴ gives strengths of the order of 2×10^7 neutrons $s^{-1} g^{-1}$ of radium for Ra- α -n sources. To produce a neutron source strong enough to average 1 neutron ns^{-1} (the hydrodynamic disassembly time) would take approximately 50 g of radium. Use of radon gas as an α source would require about 0.7 g of radon, which is still clearly unacceptable.

Other possible sources of neutrons, such as a Triga pulsed reactor, or a pulsed accelerator using a ⁹Be(d,n) reaction, would also be inadequate since, even if a high enough neutron flux could be achieved, the neutrons would strike the compressed pellet from the outside. That would be likely to initiate fission in the outer portions of the spherical mass before the desired degree of super-criticality had been reached, thus causing premature or non-uniform detonation, resulting in a fizzle yield.

As an alternative to these conventional neutron sources, a small amount of a D–T mixture could be embedded in the core of the sphere of fissionable material. Compression of the D–T core would induce thermonuclear fusion and produce 14 MeV neutrons according to the reaction



Neutrons from this reaction have been produced⁵ by the compression of a D–T pellet with a laser pulse. The rate of neutron production^{6,7} from this reaction is

$$dN/dt = N_D N_T (\overline{\sigma v})_{DT}$$

$$\text{where } N_D \approx 2 \times 10^{26} \text{ cm}^{-3}$$

$$N_T \approx 2 \times 10^{26} \text{ cm}^{-3} \quad (2)$$

$$(\overline{\sigma v})_{DT} \approx 10^{-21} \text{ cm}^3 \text{ s}^{-1}$$

are the deuterium number density, tritium number density and Maxwell–Boltzmann average of the product of

the cross section and velocity, respectively. A D–T mixture occupying 10^{-6} of the volume of the sphere of fissionable material gives 10^{12} neutrons in 10^{-11} s (the approximate neutron doubling time in the fission reaction). So the D–T fusion reaction will produce copious quantities of neutrons in the necessary time interval, and will initiate the fission reaction.

There are many advantages to this technique of initiating fusion. First, having the D–T mixture in the core of the spherical critical mass makes the initial neutron density highest at the centre of the sphere. It decreases radially outward, leading to an efficient chain reaction which develops from the middle towards the edges. Second, at the centre of the sphere there would be a pressure spike from the implosion of the sphere to critical mass; this higher pressure (and density) would make the production of neutrons by D–T fusion more efficient. Finally, since the core would be the last part of the sphere to be compressed, no neutrons would be produced until a critical (or super critical) mass was achieved. That would lead to high efficiency and would avoid the problems of a fizzle yield.

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Dissolution of powdered quartz

POWDERED quartz exhibits an initially high solubility rate but opinions are divided as to the cause. Clelland¹ argued in favour of the 'Beilby layer'—a skin of vitreous material produced during grinding; whereas Talbot² stated that the numerous small particles which were always found adhering to the larger particles of quartz offered a plausible explanation.

The fraction of quartz detectable by differential thermal analysis (DTA) becomes less³ during grinding, but the fraction can be restored to 100% by etching with hydrofluoric acid. This has been attributed to the dissolution of amorphous layers from the surfaces of the particles, leaving behind crystalline quartz. We show here, however, that when the fraction of quartz detectable by DTA is increased as a result of etching in hydrofluoric acid, the material which dissolves is crystalline and not amorphous.

A quartz sand from Chatteris, Cambridgeshire, produced a characteristic DTA inversion peak (Fig. 1a). The quartz sand was milled for 400 h in a vibration mill with a steel body containing steel balls, and iron contaminant was removed from the resultant powder using hydrochloric acid. The purified material produced a curve with a greatly reduced DTA inversion peak (Fig. 1b). The material was then subjected to two different treatments.

The first involved etching in dilute hydrofluoric acid which resulted in the dissolution of 30% by weight of the material. The residue was collected by centrifugation and produced a DTA curve with a larger inversion peak (Fig. 1c).

The second treatment involved separating the powder into a coarse and a fine fraction by dispersing the quartz in dilute hydrochloric acid (quartz is less soluble in hydrochloric acid than in water and is easier to disperse) and centrifuging the suspension to obtain a precipitate of the

coarser material, leaving the finer material in suspension. The process of dispersal and centrifugation was repeated until 30% by weight of finer material had been separated out. The coarse fraction thus obtained was subjected to DTA, and produced a curve with an inversion peak that had increased in size (Fig. 1d) compared with the original material (Fig. 1b) but, which was, perhaps, not so large as that produced by the etched quartz (Fig. 1c).

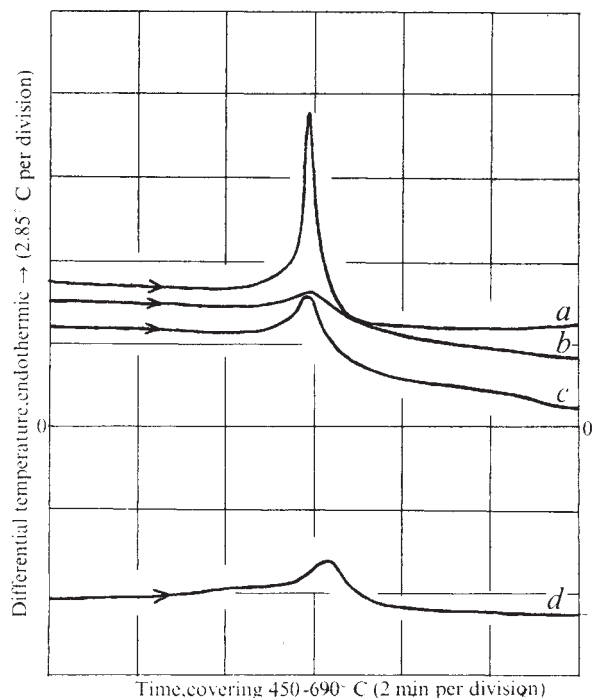


Fig. 1 Differential thermal analysis curves of an 0.285-g sample of powdered quartz heated at $20^{\circ}\text{C min}^{-1}$: *a*, original quartz sand; *b*, after grinding for a duration of 400 h; *c*, ground material from *b* after etching; *d*, ground material from *b* after the fines had been removed by sedimentation.

The fine material was subjected to two tests. First, it was suspended in hydrofluoric acid of the same strength as that used during etching. It dissolved completely within the time that had been allowed for the etching treatment. Second, a small quantity of the finest material obtained (less than $0.1\text{ }\mu\text{m}$ Stoke's diameter) was tested by X-ray diffraction (Fig. 2a) and found to consist substantially of crystalline quartz. A reference picture (Fig. 2b) for quartz milled for 3 h is also given. The fine material, shows a small amount of line broadening, but displays peak intensities almost equal to that of the reference sample.

Since the fine material dissolved completely within the time previously allotted for etching, it seems that similar fine material must have been removed by the hydrofluoric acid, leaving behind the larger particles of quartz. Furthermore, these fine particles must consist substantially of crystalline quartz (Fig. 2a and b) and are therefore not amorphous. Thus, since the removal of fine particles by sedimentation resulted in a larger DTA peak, the increase in size of DTA peak as a result of etching the powder in hydrofluoric acid must to some extent be caused by the removal of fine particles which dissolve during the etching treatment.

Does the inversion peak produced by the etched material, which is larger than that produced by the coarse material obtained by sedimentation, leave a margin of uncertainty which could be explicable in terms of the dissolution of an amorphous layer? There are three answers to this question.

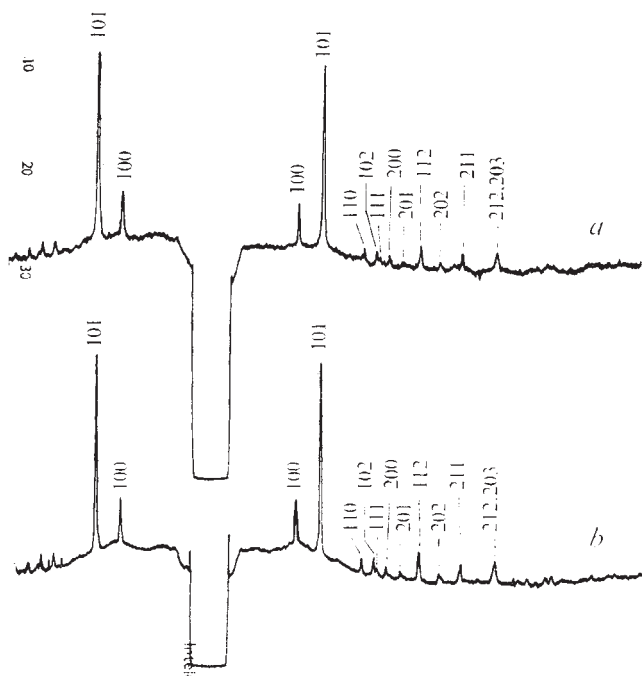
First, the processes of removing finer material by sedimentation and by etching cannot be assumed to be exactly equivalent. The powder is very difficult to disperse and sedimentation allows some very fine particles to remain attached to the larger ones. Furthermore, there is evidence (G.S.M.M. and H.E.R., unpublished) that during the grinding of quartz, 'internal subdivision' occurs resulting in the formation of grains within each particle. Such a system would be very difficult to disperse but finer grains and particles would not escape more rapid dissolution in hydrofluoric acid.

Second, the grinding of the quartz could produce residual strain⁴ within the particles. The inversion temperature of quartz is a function of pressure, and residual strain could cause the DTA inversion peak to be less pronounced if the inversion is spread over a range of temperature as a result of residual strain. When etched in hydrofluoric acid, the removal of the material under a state of strain, presumably more soluble in this condition, could produce a release of strain from the remaining quartz thus causing a more pronounced DTA inversion peak.

Third, we have found⁵ that, according to the results of density measurements, there is no evidence that amorphous material is produced, even when the quartz had been dry ground for several hundred hours until crystalline quartz could not be detected by DTA.

The density of the powder, after corrections for absorbed atmospheric moisture and impurities, remained at 2.65 g ml^{-1} during grinding, and X-ray diffraction confirmed that the powder had a crystalline structure corresponding to that of quartz. Thus, although the quartz remained in a crystalline condition during grinding, it was apparent that the inversion characteristics had become so modified that the DTA inversion peak at 573°C , normally characteristic of quartz, had disappeared. So, it seems that during etching, this finely divided quartz which we have referred to as

Fig. 2 Photodensitometer traverses of X-ray film: *a*, material with a particle size less than $0.1\text{ }\mu\text{m}$ extracted from quartz ground for 400 h; *b*, a reference curve of quartz milled for 3 h. Cu $K\alpha$ radiation, Ni filter.



'microcrystalline' and which is not easily detectable by DTA, becomes dissolved and leaves behind a coarser residue which gives a more pronounced DTA inversion peak.

Talbot⁶ states that under the abnormally severe conditions of prolonged dry grinding employed by Sakabe⁷, quartz can be converted to vitreous silica. Our evidence, however, does not support that idea.

The transformation of quartz to vitreous silica during grinding depends on whether conditions are such as to cause melting of the quartz. High temperatures and pressures during grinding are often vaguely discussed in terms of adiabatic compression, friction, electrical or other effects, but it seems⁸⁻¹⁰ that pressures of only a few tens of kilobars are produced during grinding.

Wackerle¹¹ demonstrated the conversion of quartz to vitreous silica using explosive driving systems to generate shock pressures in excess of 383 kbar. It seems unlikely that pressures of that magnitude would be produced during the process of grinding. Friction might produce temperatures of the order of 1,500°C (required to melt quartz) but only for very short periods because the average temperature of a mill during running is typically no more than 50°C; but the melting of quartz is quite slow¹² and it can be considerably overheated without melting if the temperature is rising rapidly. Thus, it seems that even under prolonged dry grinding, conditions cannot result in the transformation of quartz into vitreous silica.

Sakabe⁷ stressed the importance of the very small particles which adhere to the larger ones and he apparently never concluded that vitreous silica was produced from quartz during grinding.

One of the great difficulties of applying the concept of a 'Beilby layer' to the grinding of substances, was encountered by Clelland¹, who found that vitreous silica itself exhibited an initially high solubility when ground to a powder. The production of a skin of vitreous material over a material already vitreous does not explain satisfactorily an initial high solubility. The explanation seems to lie either in the formation of very small adherent particles, or grains within each particle, or perhaps more probably is caused by absorption of atmospheric moisture during grinding, resulting in hydrated layers. Both quartz and vitreous silica absorb atmospheric moisture during grinding¹³ and that effect seems to have been overlooked in relation to such properties as solubility characteristics or density of the powder; many researches either do not mention drying procedures, or else have used inadequate drying procedures which do not provide the temperatures of the order of 1,000°C required to liberate the combined water.

We conclude that quartz is converted during grinding into a microcrystalline form not readily detectable by differential thermal analysis. Processes such as sedimentation or etching in hydrofluoric acid, which result in the removal of the finest particles, leave behind coarser residues which behave more like the original quartz and give a more pronounced inversion on DTA curves.

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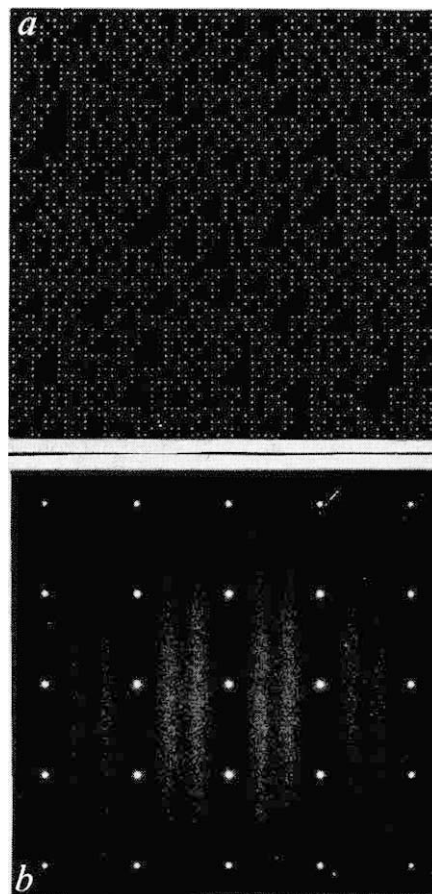
Short-range order in crystals

DURING recent studies of computer-grown crystals evidence has come to light that could possibly account for an inconsistency which has hitherto existed in the interpretation of the X-ray diffraction patterns of some real crystals which contain short-range order.

It has become increasingly evident that many crystalline materials ranging from minerals to organic molecular crystals contain some kind of short-range order. In many the interatomic forces which give rise to short-range order (either at crystallisation or subsequently through the process of diffusion) are relatively long-range, but in organic molecular crystals the forces of interaction between molecular species are short-range van der Waals' forces typically acting over only a few Ångströms. In such cases it has seemed inconsistent when the short-range order as observed by X-ray diffuse scattering apparently indicates interactions over much greater distances.

For example, during its phase transition acenaphthylene¹ assumes a structure tending to repeat every 42 Å along the *c* axis although the dimension of its unit cell at room temperatures is only 14 Å and the closely packed molecular layers are only 7 Å apart. One-dimensional models of crystal disorder² suggest that to explain such a pseudo-tripling of the cell it is necessary to consider interactions between second and possibly third

Fig. 1 *a*, Sample array; *b*, optical diffraction pattern produced by this array.



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nearest neighbouring cells, that is interactions in the range 30–40 Å.

But a two-dimensional model of crystal disorder has been developed³ and more recent studies of this model have shown that it is possible to produce a pseudo-tripling of a cell repeat in one direction when only nearest-neighbour interactions are involved.

The array shown in Fig. 1a is a small representative portion of a much larger array (500 × 500 lattice points) which was computer-grown with the model described in ref. 3. The occurrence of the two molecular species A (represented by a hole) and B (represented by a blank) at a particular lattice site is decided by consulting the nearest lattice point above and the nearest to the left. For a given combination of the species at these two lattice points (there are four: A^A, A^B, B^A, B^B) there is a constant probability that the lattice point in question is an A. In the particular example shown the probability that an A occurs after A^A is 0.0, the probability that an A occurs after B^B is 0.2, the probability that an A occurs after an A^B is 0.8, and the probability that an A occurs after B^A is 1.0.

The optical diffraction pattern of this array (Fig. 1b) has a peak in the diffuse diffraction profile at about 1/3 and 2/3 of the cell repeat indicating a tendency to a tripling of the repeat in the lattice of Fig. 1a, though this is not readily apparent simply by viewing the lattice. I emphasise that such a diffraction profile is not possible with a one-dimensional model of disorder if only nearest-neighbour interactions are involved.

It seems likely that, in real crystals where interactions in three dimensions must be considered, even more dramatic effects may be possible. Effects such as those shown by acenaphthylene may, therefore, not need the consideration of non-nearest-neighbour interactions: this possibility is being investigated by extending the present two-dimensional models to three dimensions.

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Gynodioecy in an animal

GYNODIOECY, a breeding system in which the population consists of hermaphrodites and females only, is a well known botanical phenomenon with a literature dating back at least to Darwin¹. It has never previously been reported in an animal.

A population of the sea anemone *Epiactis prolifera* Verrill, 1869, living in the rocky intertidal zone near Bodega Marine Laboratory, Sonoma County, California, was studied from April 1970 to March 1972. Of 269 animals from monthly collections examined histologically, 31 were sterile, 138 were female, and 100 were hermaphroditic (Fig. 1). This pattern of gynodioecy persisted all year round. Fertile anemones ranged from 5.8 to 35.7 mm in basal diameter. As an effort was made to include individuals of all sizes in each collection, the largest and smallest animals were represented in excess of their relative proportions in the population.

E. prolifera broods its young externally, one of 16 actinian species known to do so². Females and hermaphrodites spawn relatively few large yolky eggs which soon adhere to the parent's ectoderm, where they develop directly into juvenile anemones. When sufficiently large, juveniles move from the parent on to the surrounding substrate (D.F.D., unpublished).

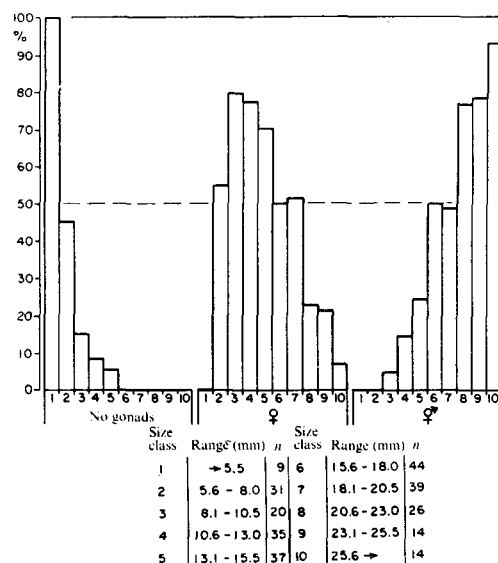
Some ideas from the botanical literature are relevant to an analysis of the breeding system of *E. prolifera*, but that part consisting of genetic analyses and models^{3,4,9} is not. Among gynodioecious plants which have been examined, individuals are genetically either hermaphroditic or female. In *E. prolifera* it seems that sex determination is virtually identical in all individuals so that animals begin their reproductive lives as females, and most that survive sufficiently long become hermaphrodites. Thus gynodioecy in *E. prolifera* is serial and developmental, whereas in plants it is fixed and genetic.

A major advantage of gynodioecy—its ability to adjust the degree of heterozygosity in a population^{3,4,9}—obtains only if the species is at least potentially self fertile. There is no reason to dismiss this possibility in *E. prolifera*, where ripe ova and spermatozoa frequently occur in the same animal simultaneously (D.F.D., unpublished). If hermaphroditic individuals were facultatively self fertile, advantageous characters, including those which enabled them to survive sufficiently long to develop testes, could be fixed in the genome of the population by homozygosity. Under favourable environmental conditions, longevity would increase, raising the proportion of hermaphrodites in the population and thereby the potential for inbreeding. When conditions were such that few anemones survived long enough to develop testes, inbreeding would decline, thus increasing the proportion of heterozygous offspring with greater genetic flexibility to meet the stressful conditions.

Genetic factors allowing animals to survive long enough to develop testes and producing gynodioecy in *E. prolifera* are favoured numerically, even if selfing is impossible. Offspring of females receive half their genes from hermaphrodites; those of hermaphrodites receive all of theirs from hermaphroditic parents, one if by selfing and two if by crossing. Thus the genetic contribution to the next generation from hermaphrodites, no matter how few, cannot be less than 50%. It is usually greater because hermaphrodites will have already produced offspring as females, and because larger anemones brood more young (D.F.D., unpublished).

It is crucial to the species' success that the prevalence of egg-producing individuals in the population be as great as possible, for fecundity of *E. prolifera* is restricted by brood space. The strategy of having barely enough males to fertilise the ova produced by a population consisting largely

Fig. 1 The relationship between size and gonadal state of 269 *E. prolifera* collected approximately monthly over a period of 2 yr. Size classes are based on pedal disk diameter at the time of collection.



of females might be genetically too inflexible. The extreme in maximisation of brood space would be for all members of the population to produce eggs, some or all of them producing sperm also. (Carlquist¹⁰ reasons similarly regarding gynodioecious plants.)

This last possibility, simultaneous hermaphroditism, occurs in the only other species of externally-brooding actinian to have been studied¹¹, called *E. prolifera* at that time, this species is now identified as *E. japonica* (T. Uchida, personal communication). Selection against sperm production beyond what is necessary to fertilise sufficient ova to fill the brood space by restricting it to certain members of the population is a possible evolutionary pathway from simultaneous to gynodioecious hermaphroditism. The larger anemones might have been favoured as sperm-producers since they had proved best adapted by virtue of their longevity, and because proportionately less of the energy and material budgeted for gametes could be used by them for sperm than by smaller animals.

Developmental gynodioecy in self fertile species would favour reproduction by only the fittest colonists of new habitats, maximising the chances of survival of succeeding generations. If a hermaphrodite were the founder, it could begin to establish a population immediately. If the animal had not yet attained hermaphroditism, it could begin to reproduce successfully only after having proved its fitness for the new circumstances by surviving in them. Even assuming *E. prolifera* can self, however, it must rarely survive being swept into a new site, so this advantage probably had little to do with the evolution of gynodioecy in this species.

Developmental gynodioecy is an adaptable breeding system. Theoretically, the genetic diversity of gametes is as great as in simultaneous hermaphrodites because virtually all individuals eventually produce sperm as well as ova. Excessive sperm production can be prevented by evolutionary adjustment of the time of onset and extent of testicular development to optimise the sperm: egg ratio of the population. At any one time, the same ratio can be attained as in dioecious species but with greater genetic diversity of sperm because more hermaphrodites are required to produce as many sperm as can be produced by a given number of purely male individuals of the same size.

The genetically-fixed gynodioecy of plants apparently evolved as a mechanism for enhancing outbreeding among normally self fertile hermaphrodites. The developmental gynodioecy of *Epiactis prolifera* probably evolved as a means of increasing the number of brooders (egg-producers) in a population with limited fecundity to a maximum, and possibly also of enhancing inbreeding among normally cross fertile individuals.

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Evidence for a Y chromosomal contribution to an aggressive phenotype in inbred mice

IN man the XYY chromosome complement has been reported by some authors to be associated with abnormal tallness, mental deficiency and sociopathic, aggressive behaviour¹⁻⁴. The interpretation of this literature is controversial. Furthermore, any genetic variation(s) of the Y chromosome which affect(s) aggressive behaviour among chromosomally normal males could mask the correlation between a supernumerary Y and this behaviour⁵. If such variation exists, then in any population the correlation between the XYY karyotype and aggression would depend on the distribution of Y chromosomes having varying degrees of predisposition towards aggressive behaviour. Our investigations on the developmental genetics of fighting behaviour in mice support the hypothesis that there may be heritable variations of the Y chromosome which are associated to varying degrees with some types of aggression, and that this could account both for the lack of unusual aggressive behaviour in many individuals with an extra Y and for its occurrence in other XYY males.

In an initial study, one of us⁶ showed that male DBA/1/Bg mice were more aggressive than C57BL/10/Bg males and that in their reciprocal hybrids, those F₁ males sired by DBA/1/Bg were more aggressive than those sired by C57BL/10/Bg. These findings, suggested to us that the Y chromosome of the DBA/1/Bg makes an incremental contribution to intermale aggressiveness. We report here the original data⁶ and a replication entailing a larger sample.

Intraspecific aggression was measured in encounters between pairs of male mice isolated from the time of weaning at 29 d of age until testing at 50 d of age when they were sexually mature. Pasteurised food and water were available *ad libitum*. Water was acidified (pH 2.5) and chlorinated (12-18 p.p.m.). Lighting was on a 12 h light: 12 h dark diurnal cycle with lights on at 0600. The mice were tested in a laboratory cage (16 × 26.5 × 11.5 cm) covered by a 6.4 mm wire mesh. The cage was divided by an opaque partition. Subject animals were placed on either side of the partition which was removed after a 5-min adaptation period. Trials were limited to 10 min during which behavioural records were kept for each 20 s interval. Each pair was tested daily for three consecutive days. Agonistic acts recorded consisted of tail rattling, wrestling, flank biting, chasing or full attack⁷⁻⁹. Any aggressive act by one mouse during a 20-s interval was scored as '1'; if both mice reacted aggressively during the same period, it was scored '2'. Aggression scores were obtained by summing the pair scores over the three trials and averaging these scores for each genotype.

Table 1 Reciprocal effects on aggressive behaviour

	Initial*		C57BL/10 hersFat
	DBA/A Fathers		
DBA/1 Mothers	34.3 ± 5.1† (10)‡		21.5 ± 5.5 (10)
C57BL/10 Mothers	34.0 ± 3.0 (10)		5.0 ± 2.1 (10)
DBA/1	B10D1F1	D1B10F1	C57BL/10§
34.3	34.0¶	21.5	5.0
	Replication		C57BL/10 Fathers
	DBA/1 Fathers		
DBA/1 Mothers	35.4 ± 4.6 (20)		10.7 ± 2.2 (42)
C57BL/10 Mothers	19.8 ± 3.0 (43)		7.3 ± 2.3 (22)
DBA/1	B10D1F1	D1B10F1	C57BL/10
35.4	19.8	10.7	7.3

* The initial study was carried out at the University of Chicago between 1967 and 1968 and the replication was done at the University of Connecticut between 1972 and 1973.

† $\bar{X} \pm$ s.e.m.; maximum possible aggression score was 180 for both studies.

‡ Number of male pairs tested.

§ Neuman-Keuls analysis of aggression score means; $P < 0.05$ (after Winer¹⁰).

¶ Underlined means are statistically equivalent.

Table 2 Matherian scaling of aggression scores

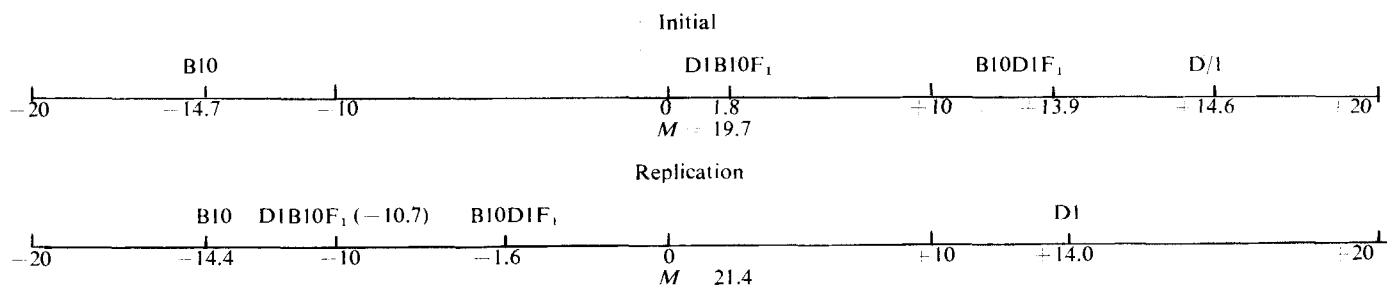


Table 1 indicates the aggression score obtained in the two experiments. The scores for each parental strain and thus for the mid-parental values are essentially unchanged. In the initial study, a marked but non-significant difference ($P < 0.10$) was noted between the two reciprocal F_1 mice. This difference attained significance in the replication ($P < 0.05$; Student's t test). Analysis of variance for all four genotypes indicates a highly significant F statistic for both samples ($P < 0.001$). A Neuman-Keuls analysis designates the significant differences between genotypes (Table 1). In the initial study, the score of the B10D1F₁ is similar to that of the DBA/1 strain; whereas in the replication, it is closer to that of the mid-parent. In the same study, the score of the D1B10F₁ is similar to that of the mid-parental value; whereas in the replication, it is closer to that of the C57BL/10/Bg strain. These relationships can be seen more clearly on a Matherian scale (Table 2) where the aggression score of each genotype is represented as a deviation from the mid-parental value¹⁰. In both samples, the reciprocal differences are in the same direction, and their magnitude, measured as deviations from the mid-parental values, are essentially the same.

A possible autosomal contribution to aggressive behaviour was detected in the original study⁶, in a study by Eleftheriou *et al.*¹¹, but not in the present one. The factors accounting for this discrepancy are possibly that the animals used in the replication, though of the same substrain (Bg), were at least 20 generations removed from those used in the initial study. They were also maintained under specific pathogen-free conditions, while the original animals were housed in a conventional colony. That the Y chromosomal contribution could be replicated in the hands of a second investigator under somewhat different environmental and testing conditions suggests that the latter effect is more robust.

Since the DBA/1/Bg mice are more aggressive than the C57BL/10/Bg mice, and since the magnitude of the F_1 scores is associated with that of the male parent, it is a reasonable hypothesis that the reciprocal differences in aggressiveness are the result of the reciprocal differences in the origin of the Y chromosome, as previously suggested¹². Because the more aggressive hybrids developed in the uterine and postnatal environment of the less aggressive C57BL/10/Bg strain, these maternal conditions seem to make no major contribution to the aggressive behaviour of these strains and hybrids.

Although there seems to be an incremental effect of the Y chromosome derived from DBA/1/Bg mice on aggressive behaviour, our studies indicate that the Y chromosome of the aggressive DBA/2/Bg strain does not potentiate intermale fighting when analysed in similar crosses. Also, in reciprocal crosses between CFW and A/J mice, Southwick¹³ reported that F_1 mice sired by the more aggressive strain (CFW) were not more aggressive than their reciprocal F_1 generation. Thus, the variation in intermale fighting in mice is very likely under the control of a variety of genetic factors, including the Y chromosome.

These results add to the growing evidence¹⁴⁻¹⁷ which suggests

that the Y chromosome, in at least one mammalian species, is not inert and that extrapolating from mouse to man, there may be active genes on the Y for traits other than hairy ears, foot ulcers and radio-ulnar synostosis¹⁸. Our hypothesis thus places constraints on inferring behavioural potential from the XYY karyotype of man or other mammals.

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Response of flying mole crickets to three parameters of synthetic songs broadcast outdoors

LARGE numbers of flying crickets can be attracted to outdoor loudspeakers broadcasting male calling songs¹. Consequently we have been able to investigate the features of calling songs that are important for attraction. Previous investigators have used laboratory-maintained crickets in acoustical environments significantly different from outdoors²⁻⁷. Furthermore, they have studied thoroughly no more than one or two of the important signal parameters. We have studied outdoors the responses of free-flying mole crickets, *Scapteriscus acletus*, to synthetic call-

ing songs systematically varied in these three parameters: carrier frequency, pulse rate and intensity. Mole crickets came in greatest numbers when we broadcast, at unnaturally high intensities, carrier frequencies and pulse rates like those of the natural song.

All experiments were conducted near Gainesville, Florida, during 1972 and 1973. During the tests soil temperatures were $24 \pm 4^\circ\text{C}$, air temperatures, $25 \pm 5^\circ\text{C}$, and relative humidity, $70 \pm 20\%$ (ref. 8). We used three independent broadcasting systems, each with its own cricket-trapping funnel. A fourth funnel, without a speaker, was the control. Two test sounds and a standard sound were broadcast simultaneously. Test sounds were synthetic songs (artificial sounds synthesised and tape-recorded in the laboratory) varied in intensity, carrier frequency and pulse rate (that is, rate at which the carrier was turned on and off). Equal durations of pulses and pulse intervals were maintained. For frequency experiments (1972), the pulse rate and intensity were held constant at 60 pulses s^{-1} and 100 dB sound pressure level (SPL) (at 15 cm; reference SPL, $2 \times 10^{-5} \text{ N m}^{-2}$). Similarly, in pulse rate experiments (1973), the frequency and intensity were held constant at 2.7 kHz and 100 dB. In intensity experiments (1973), frequency and pulse rate were constant at 2.7 kHz and 55 pulses s^{-1} . The standard sound was 2.7 kHz and 100 dB. It was changed from 60 pulses s^{-1} in 1972 to 55 pulses s^{-1} in 1973 to approximate more closely the natural song (that is, the actual calling song of males in the field) at 25°C . The number of mole crickets trapped at each test sound was expressed as a percentage of the number caught at the standard sound. Trials with less than 10 crickets in the standard were discarded. Each test sound was used on at least two nights. We have described the trapping equipment and the broadcasting techniques before¹.

The control funnel generally caught no mole crickets and never had more than 1.5% of the number of adults in the other three funnels. In frequency and pulse rate trials the number of trapped mole crickets varied fivefold about a mode (Fig. 1). For every 6-dB increase in sound level up to 106 dB, our catch approximately doubled (Fig. 2). In addition to comparing seven pulse rates, we investigated whether any amplitude modulation at all was necessary for phonotaxis of *S. acletus*¹⁰. We made four trials comparing a continuous tone (no amplitude modulation) of 2.7 kHz with the 1973 standard sound (tone turned on and off 55 times per second). Because 270 *S. acletus* were captured at the standard sound, 27 at the continuous tone, and none at the control, amplitude modulation must be an important, but not essential, feature.

Several features of male calling songs are known or suspected to cause species-specific responses in crickets²⁻⁷. For mole crickets, Bennet-Clark¹¹ suggested that carrier frequency might

Fig. 1 The response of flying *S. acletus* to synthetic sounds that were systematically varied in frequency (a) and pulse rate (b). The relative response is a percentage of those captured at the standard sound (circle and cross) (see text). Vertical bar is 2 s.e. on either side of the mean. Narrow vertical line is range. In 16 frequency trials, 416 individuals were trapped; in 24 pulse rate trials, 843 were trapped.

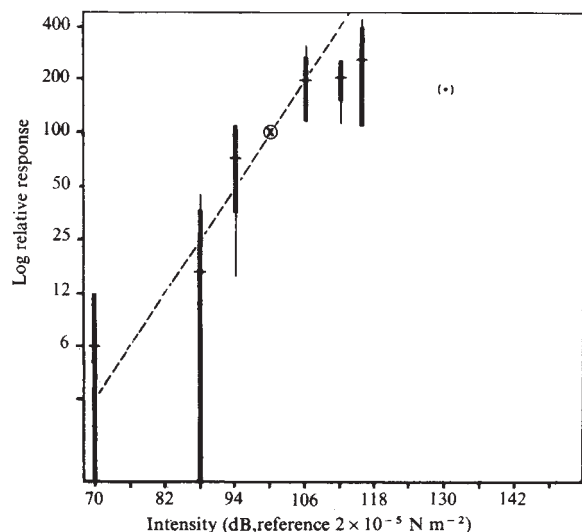
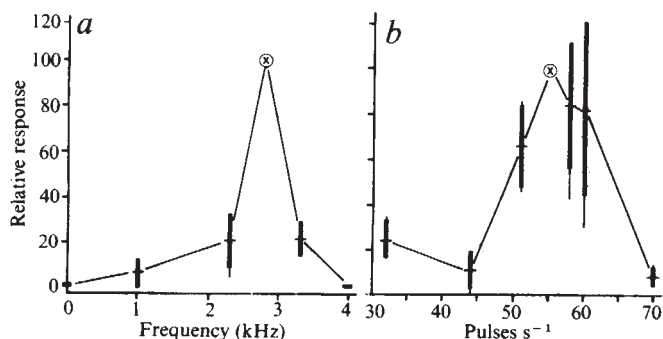


Fig. 2 The response of flying *S. acletus* to various intensities. Relative response is a percentage of those captured at the standard level (circle and cross, 100 dB). (●) Represents result of a single trial with a different speaker. The dotted line represents a doubling of the number for each 6 dB increase in sound pressure level. Vertical bar is 2 s.e. on either side of mean. Narrow vertical line is range. In 33 intensity trials, 1,606 individuals were captured.

be important to flying females. Our data (Figs 1 and 2) demonstrate that both carrier frequency and pulse are important to species-specific phonotaxis in *S. acletus*. This insect (Orthoptera, Gryllotalpidae) should be able to separate its own song from songs of most other crickets by pulse rate (Fig. 1); however, there are crickets occurring in the same habitat with pulse rates overlapping those of *S. acletus*. *S. acletus* could distinguish the songs of its own males from the songs of these other crickets by carrier frequency, since carrier frequency of these songs is higher than in *S. acletus*¹.

Doubling of catch for each 6-dB increase in sound level agrees with the following model. Sound waves radiate in all directions from loudspeakers, and sound pressure is generally halved (that is, drops 6 dB) for each doubling of distance from a sound source. Whatever the shape of the sound field, its diameter would double for each 6-dB increase in sound level. To orientate to a calling song, the flying adults must first encounter an appropriate sound above a threshold pressure. If mole crickets fly in a single plane and maintain straight courses, the catch should double for each doubling of diameter (not the area) of the sound field. The failure of doubling to continue with each increase of 6 dB beyond 106 dB could result from the crickets being repelled by such high intensities or from an inability to orientate owing to saturation of their auditory organs.

The synthetic songs that trapped maximal numbers of mole crickets had pulse rates and frequencies like those of the natural song but the catch was increased 30-fold at intensities 38 dB or more above natural intensities, which average 68 dB⁹. Such attraction may prove useful in control of these agricultural and turf pests.

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Ripening of tomato fruits at reduced atmospheric and partial oxygen pressures

STORAGE at reduced atmospheric pressure and low temperature is used to retard the post-harvest senescence of fruits, vegetables, cut flowers, and cuttings¹⁻³. The effect of this low pressure storage (LPS) has mainly been ascribed to reduction of the internal concentration of the senescence-accelerating gas ethylene⁴, although occasionally effects of a lowered partial oxygen tension (P_{O_2}) have also been reported⁵⁻⁷. To determine the contribution of the reduction in P_{O_2} to the total effect of LPS on the retardation of post-harvest senescence, we studied the course of ripening of tomato fruits at different atmospheric and partial oxygen pressures, with and without absorbing the ethylene produced. We report that both the onset and the rate of the ripening process are determined by the oxygen pressure, irrespective of the atmospheric pressure and the absorption of endogenously evolved ethylene.

Tomato fruits of the Jupiter cultivar were grown under greenhouse conditions and carefully selected from the same position at comparable clusters. Samples of 15 mature green fruits were placed in 21-l desiccators over dry lime to absorb the carbon dioxide produced. Part of the desiccators also contained a 3% potassium permanganate solution in 1 N sulphuric acid, to absorb the ethylene evolved. The ethylene concentration in the desiccators was measured daily using a gas chromatograph. After gas sampling the desiccators were flushed to refresh the atmosphere completely. Gas mixtures were applied from cylinders and the atmospheric pressure adjusted by suction. When the fruits were in their climacteric stage, respiratory oxygen consumption never reduced P_{O_2} below 0.18 and 0.037 at initial P_{O_2} values of 0.21 and 0.04, respectively. The respiration rate at $P_{O_2} = 0.1$ was still equal to that at $P_{O_2} = 0.2$, but at a pressure of 4% it must be considerably reduced. The onset and rate of ripening were followed daily using visual colour determination, and at the table-ripe stage the softness of the fruits was also measured⁸. The weight loss never exceeded 2%. Table 1 summarises the results of an experiment at 19° C, under diffuse light from fluorescent tubes.

The normal period taken to ripen, 7 days, is delayed by pure oxygen at $P_{O_2} = 1.0$; also the lycopene synthesis is disturbed, the ripe soft fruits becoming intensely orange but not red. At about $P_{O_2} = 0.2$, however, the presence of other atmospheric components fails to affect ripening, the fruits ripening in 7 days both at normal and at reduced atmospheric pressure, whether or not the ethylene is absorbed. Even at 19 cm Hg and in the presence of permanganate solution, the ethylene level does not limit the rate of ripening, whereas at the reduced oxygen level of 5%, ripening is considerably retarded, even without absorption of ethylene.

Table 2 shows an experiment at two temperatures, 19° C and 13° C. Again, at the normal P_{O_2} of 0.2 the usual ripening periods of 9 and 17 days occur at 19° C and 13° C, respectively,

Table 2 Rate of ripening of Jupiter tomato fruits, from the mature green to the table-ripe stage, at different atmospheric conditions at two temperatures

cmHg	Atmosphere	P_{O_2}	KMnO ₄	Days to ripeness	
				19° C	13° C
76	Air	0.2	+	9	17
			—	9	17
15	O ₂	0.2	+	9	16
			—	9	17
15	Air	0.04	+	24	28
			—	24	28
76	N ₂ +O ₂	0.04	+	29	32
			—	29	32

regardless of the total atmospheric pressure and the absorption of ethylene. In the presence of permanganate solution, the level of ethylene never exceeded $2 \mu\text{l l}^{-1}$ and was usually much less than $1 \mu\text{l l}^{-1}$. Without ethylene absorption, the ethylene level at 20% oxygen surpassed the threshold value (ref. 9 $5 \mu\text{l l}^{-1}$) for ripening of tomato fruit within 2 days; at the reduced oxygen level of 4%, on the other hand, the ethylene level always remained below $4 \mu\text{l l}^{-1}$, mostly below $2 \mu\text{l l}^{-1}$, and the ripening was considerably retarded in all cases. The lack of oxygen is the overriding factor at $P_{O_2} = 0.04$, determining the rate of ripening. At normal atmospheric pressure the ripening was even more delayed than at reduced pressure.

That not only the rate, but also the onset, of ripening is delayed at a low partial oxygen pressure is shown in an experiment with earlier harvested fruits (Table 3). In air at normal pressure, the fruits started ripening after 4 days and reached the pink stage in 8 days. The reduced partial oxygen pressure of 4% postponed the onset of ripening considerably, again at an atmospheric pressure of 76 cm more than at 15 cm. The conclusion that oxygen is the limiting factor at this concentration, rather than ethylene, is supported by the fact that the presence of the permanganate solution is irrelevant.

Table 3 Delay of onset of ripening, to the first sign of colour breaking (stage 2), at different atmospheric conditions at 19° C

cmHg	Atmosphere	P_{O_2}	KMnO ₄	Days to stage 2
76	Air	0.2	+	4
			—	4
76	N ₂ +O ₂	0.04	+	16
			—	16
15	Air	0.04	+	10
			—	13

We have therefore confirmed the usefulness of LPS in delaying post-harvest senescence. Contrary to earlier indications¹, however, we have clearly demonstrated that, at least for the commodity and conditions used, the effect of LPS is not primarily caused by a reduced ethylene concentration, but rather by the low partial oxygen pressure. In this respect LPS acts in a similar way to the well established method of controlled atmosphere storage, in which senescence is retarded by storage in an atmosphere reduced in oxygen and enriched in carbon dioxide by the respiratory activity of the commodity¹⁰⁻¹². Among the advantages of LPS are the more readily established atmospheric conditions and the continuous removal of carbon dioxide and ethylene. The reduced oxygen pressure may affect the onset and rate of ripening by inhibiting the biosynthesis or activity of ethylene^{12,13}, or by limiting the respiratory activity or interfering with the oxidation of, for example, indoleacetic acid¹⁴.

We also conclude that the concentration of endogenously evolved ethylene in the atmosphere has no effect on the onset and the rate of ripening, if it is not allowed to accumulate excessively. The ethylene produced in ripening tissue may well exert its physiological effect mainly during its passage, within the cell, from the site of biosynthesis to the intercellular space. The concentration of endogenous ethylene in the intercellular space is thus an indicator rather than an effector of ripening

Table 1 Rate of ripening of Jupiter tomato fruits, from the mature green to the table-ripe stage, at different atmospheric conditions at 19° C

Pressure (cmHg)	Atmosphere	P_{O_2}	KMnO ₄	Days to ripeness
76	O ₂	1.0	+	13
			—	13
76	Air	0.21	+	7
			—	7
19	O ₂	0.25	+	7
			—	7
19	Air	0.05	+	27
			—	27

and its reduction by low atmospheric pressure or absorption is not effective in regulating senescence.

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Blue-green algae associated with ascidians of the Great Barrier Reef

IN the rich biota of reef communities, one of the best known symbiotic relationships is that of dinoflagellates known as "zooxanthellae" with corals and giant clams^{1–3}. In contrast, the presence of algae in ascidians (sea squirts: Phylum Chordata, Subphylum Tunicata), although known for many years, has been studied very little. It is known that the association is confined to tropical ascidians in the family Didemnidae, but even the phylum to which the algae belong, or indeed whether the green cells in question are algae at all, has remained uncertain. During a recent expedition of the RV Alpha Helix to the Great Barrier Reef, we encountered several species of colonial ascidians containing large numbers of bright green, spherical cells. We have established by optical and electron microscopy that these green cells are blue-green algae; their association with primitive chordates represents a considerable extension of the known host range of these prokaryotes.

Our collections were made between March 25 and May 13, 1973, on reefs surrounding Lizard, Nymph and Turtle Islands off the north-east coast of Queensland (14°40'S, 145°30'E). Of the species of ascidians we collected five belong to the Didemnidae (Patricia Kott Mather, personal communication). We found that three of these [*Didemnum ternatanum* (Gottsch.), *Diplosoma virens* (Hartmeyer), and *Lissoclinum molle* (Herdman)] contained large numbers of algae. *Didemnum ternatanum* is a brown-coated, usually urn-shaped species 1–3 cm in height, while *Diplosoma virens* and *Lissoclinum molle* are blue and green encrusting forms, respectively. In all three ascidians the seawater strained of its plankton by the zooids collects in the labyrinthine central cloacal cavity of the colony, from which it is expelled through common excurrent pores. The algae are located extracellularly within cloacal pockets and folds around the zooids, or appressed to strands of the cellulose-like matrix or test that span the cloacal cavity (Fig. 1).

Earlier observations on the algae of didemnid ascidians had identified them as either zooxanthellae^{4,5} or zoochlorellae⁶, the latter category including various green algae associated with animals⁷. We concluded from light microscopy, however, that the algae in our ascidians are blue-greens, since they lack discernible nuclei and form new cells by wall ingrowth and constriction. These algae are bright green, their absorption spectra indicating the presence of chlorophyll *a* and little or no phycocyanin and phycoerythrins (T. Akazawa, personal communication). All three algae are unicellular and spherical, but in other respects differ sufficiently to be readily distin-

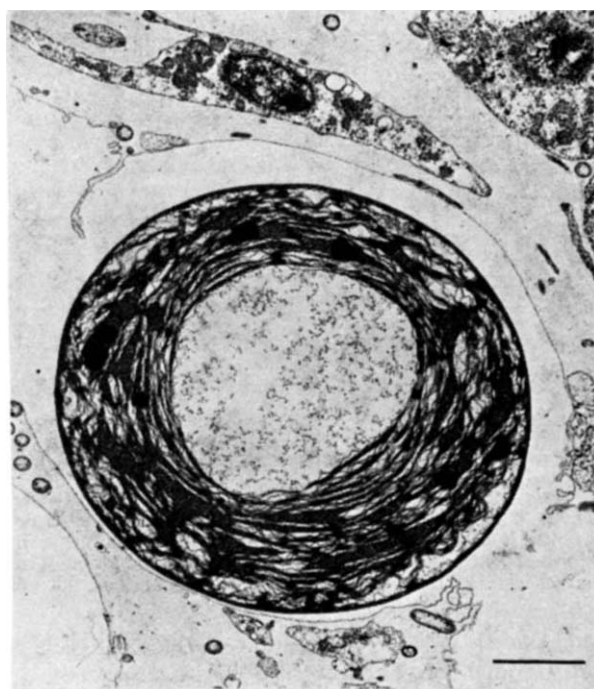


Fig. 1 Thin section through a coccoid blue-green alga lying in the cloacal cavity of the colonial tunicate *Diplosoma virens*. This electron micrograph illustrates the concentric lamellae, polyhedral inclusions and clear central area of the alga. The alga resides in a pocket formed by the test of the colony. Cells of a zooid can be seen at top right. Bacteria are embedded among the cellulosic fibrils of the test at lower left and elsewhere. Scale bar, 2 μ m.

guishable from one another. The algae of *Diplosoma virens* and *Lissoclinum molle* usually contain a clear central region not seen in the algae of *Didemnum ternatanum*. Also, the algae of *D. virens* range in diameter from 7–15 μ m whereas the algae from *D. ternatanum* and *L. molle* average about 20 μ m.

The three algae have a basically similar ultrastructural organisation but differ in detail. The cells lack mitochondria and other eukaryotic organelles, and their fine structural features are characteristic of blue-green algae (Figs 2 and 3a). Since the fine structural features are quite constant between algae from different collections of the same species of ascidian, a single micrograph is usually sufficient to enable identification of the alga in terms of its ascidian host (compare Figs 2 and 3b). On the basis of classical taxonomic features determined with the optical microscope, the algae from formalin-preserved colonies of all three ascidians have been identified as the single blue-green species, *Anacystis aeruginosa* (Zanard.) Drouet & Daily (Francis Drouet, personal communication). But, the consistent fine structural differences suggest that the three algae may represent different species, each associated with its particular species of ascidian.

There are reports of the presence of algae-like cells in the cloacal cavities of colonial ascidians. Herdman⁸ in 1906 mentioned their presence in the cloacae of *Diplosoma virens* and *D. viride* (= *D. virens*) from Ceylon. On the Yonge expedition to the Great Barrier Reef in 1928–29, Hastings¹ observed brownish algal inhabitants in the cloacal cavities of three didemnid ascidians, including *D. virens* and *Lissoclinum molle*. The algae were identified as zooxanthellae by Yonge¹ whose identification was corroborated by Smith⁹ from preserved specimens. Smith also pointed out, however, that in a collection of *D. virens* from Ceylon the algae, being green, were possibly zoochlorellae. More recently, Tokioka⁶ examined ascidians from the Pacific Ocean in the United States National Museum and found that seven didemnids harboured algae, all of which were identified as zoochlorellae. Thus there are several didemnid

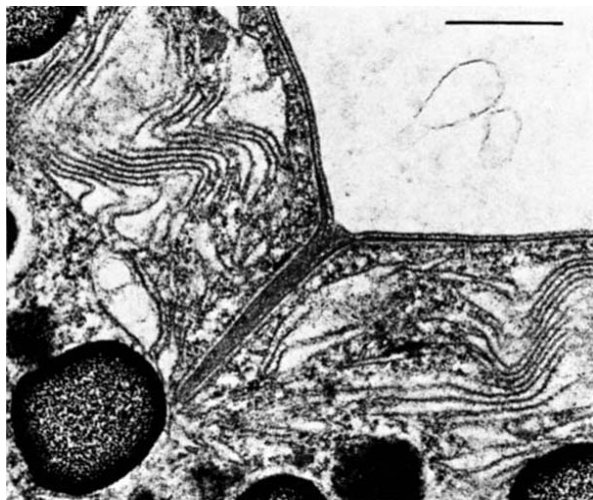


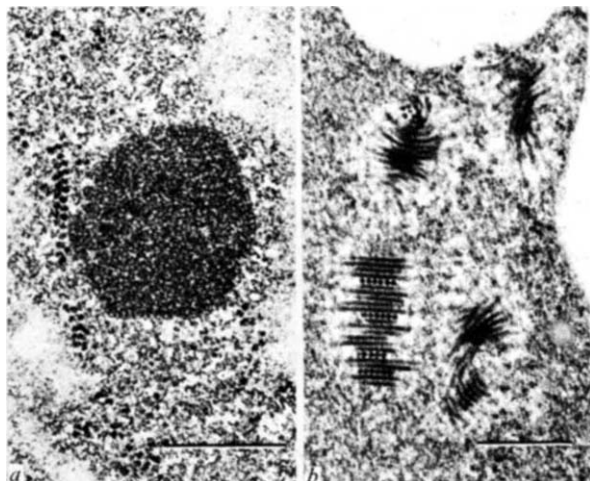
Fig. 2 Ingrowth of the wall bounded by the plasmalemma during cell division by constriction in the alga from a mature colony of *Didemnum ternatanum*. The osmiophilic globules are characteristic of the algae in this ascidian. Scale bar, 0.4 μ m.

ascidians in which zooxanthellae and zoochlorellae have been reported from different collections of the same species.

We conclude from our observations that some or all of the zoochlorellae reported in ascidians by past workers may have been blue-green algae. In any event it seems clear that in several cases a particular ascidian species is capable of harbouring either of two quite different algae. Thus *Diplosoma virens* and *Lissoclinum molle* collected at Low Isles on the Yonge Expedition contained zooxanthellae, while the same species collected by us approximately 115 miles north of Low Isles (and 45 years later!) contained blue-green algae. There is no evidence that mixtures of the two kinds of algae occur. In examining thousands of algae and the tissues of many zooids in our didemnid ascidians, we have not found a single zooxanthella, although zooxanthellae exist in enormous numbers in the nearby corals and clams.

Although earlier reports have established the presence of either zooxanthellae or zoochlorellae in different collections of *Trididemnum cyclops*⁴⁻⁶, the presence of algae may not be necessary for the existence of this ascidian at least, as we found flourishing colonies of this species growing without algae on the underside of a coral rock. Whether the ascidians utilise photo-

Fig. 3 a, Particles suggestive of ribosomes helically disposed in a polysome lie adjacent to a polyhedral inclusion in an alga from *Lissoclinum molle*. All of these structures are common to the algae of the three ascidians. Scale bar, 0.3 μ m. b, Characteristic paracrystalline arrays of electron opaque structures arranged as rods and arcs in an alga from *Lissoclinum molle*. Scale bar, 0.3 μ m.



synthetic products of the algae, as do clams and corals, has not yet been established. Tests for nitrogen fixation by the algae were negative (R. H. Burris, personal communication).

Previously, blue-green algae in the marine environment have been identified as symbionts only of certain diatoms¹⁻³ and a very few marine invertebrates, among which are certain sponges⁹⁻¹². The association we observed is therefore of special interest since it greatly extends the host range of blue-green algae to include primitive members of the Chordata, and thus represents an association with marine animals considerably higher in the evolutionary scale than hitherto recognised.

Our formalin-preserved ascidians have been deposited in the Queensland Museum; we thank Dr Patricia Kott Mather for their identification. We also thank Mr J. E. Burris and Drs T. Akazawa, J. S. Bunt, R. H. Burris, I. R. Price and D. D. Randall for assistance in collecting, and Dr L. Muscatine for advice. This work was supported by grants from the National Science Foundation to the Scripps Institution of Oceanography for operation of the Alpha Helix Research Program, and by a grant from the National Science Foundation to the senior author.

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Heteroantagonism observed in mixed algal cultures

RUSSELL¹ has referred to the curious lack of interest shown by marine ecologists in species competition, especially in view of the suitability of existing cultural techniques for investigating this problem. Certainly much more experimental work using mixed cultures has been carried out by examining competition in planktonic algae²⁻⁷ than in benthic algae⁸. This is perhaps surprising in view of the wealth of information, particularly that derived from studies on the algal colonisation of cleared or new substrata, which suggests that competition factors also play active roles in benthic community structuring and may even, as deduced by Den Hartog⁹, influence zonation. Particularly interesting in this respect are the growing number of reports¹⁰⁻¹⁴ that polyphenols ('tannins') are actively liberated from the surfaces of brown algae, and that these compounds possess both antibacterial and antialgal properties¹⁵⁻²⁰. Indeed, the fucosan-vesicles or physodes commonly found in brown algal cells have been attributed the function of polyphenol storage²¹⁻²³. Although the antifouling properties of these secretions have been recognised^{7,16,17,19,20}, very little is known of their ecological influence beyond the surface of the host. Here I report on some observations of mixed cultures of the brown crustose alga *Ralfsia spongiocarpa* Batt. and the two crustose red algae *Porphyrodiscus simulans* Batt. and *Rhodophysema elegans* (Crouan frat. ex J. Ag.) Dixon.

Both the *Ralfsia* thalli and the two crustose red algae were collected from the rocky sides of low-tide-level pools at Peveril Port, Swanage, Dorset; laboratory cultures were established using unispores²¹ and tetraspores, respectively. The abundance

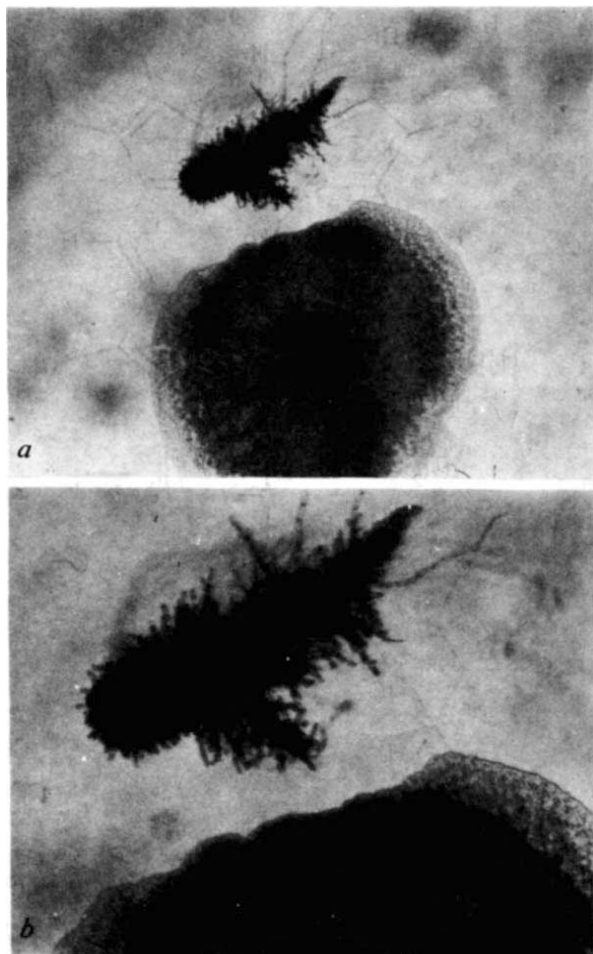
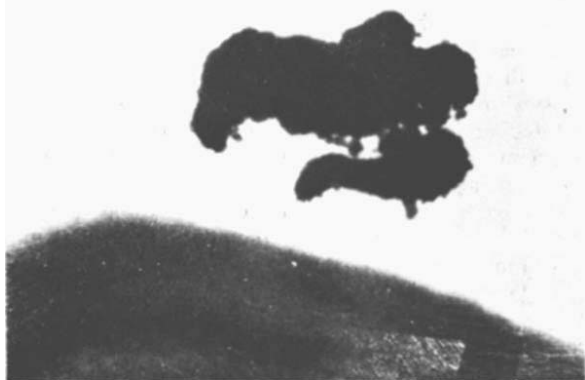


Fig. 1 *a*, Part of a 6-week-old cultured crust of *Rhodophysemma elegans* showing inhibition of horizontal growth on the side adjacent to a knot-filament of *Ralfsia spongiocarpa* ($\times 64$). *b*, Closeup of (*a*) ($\times 136$).

of the *Ralfsia* under the field conditions²⁵ suggested that the thalli had competitive advantages. Cultured crusts of the two red algae were later 'seeded' around their perimeter with small portions of cultured *Ralfsia* 'knot-filament' thalli (for a description of this phase in the life history see ref. 25). Care was taken to ensure that the *Ralfsia* thalli did not come into contact with the crusts but were allowed to form a weak attachment to the floor of the Petri dish a short distance away from them. 'Control' cultures were also set up in which an approximately equal amount of red algal 'knot-filament' growth was placed adjacent to cultured crusts of the same species. All cultures were placed at 10° C, 8–16-h day conditions using Gro-lux fluorescent tubes at

Fig. 2 Part of a 4-month-old cultured crust of *Porphyrodiscus simulans* showing inhibition of horizontal growth on the side adjacent to a knot-filament of *Ralfsia spongiocarpa* ($\times 45$).



an intensity of 1,000 lx. The culture medium was replenished each week.

Within approximately 10–14 d the growth of the two red algal crusts was markedly inhibited on the sides adjacent to the *Ralfsia* thalli (Figs 1 and 2). The broad monostromatic zone, typically produced in the other regions of the germlings and in the control cultures by outer, actively dividing meristematic cells, was absent and polystromatic crust formation was evident right up to the thallus margin (Fig. 3).

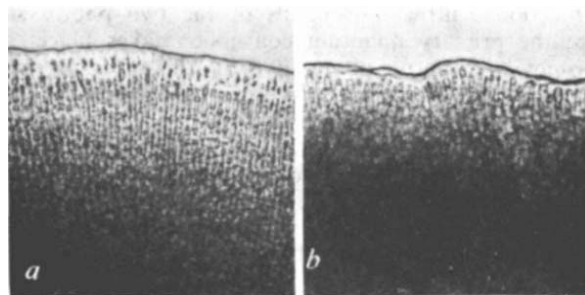


Fig. 3 *a*, Surface view of normal, actively growing margin of a cultured crust of *Porphyrodiscus simulans* ($\times 201$). *b*, Surface view of the margin of the same crust adjacent to the *Ralfsia* thalli ($\times 173$).

In view of the rapid nature of the response, it is unlikely that this growth inhibition effect was caused by differential nutrient uptake by the two adjoining algae. The evidence strongly suggests that an antibiotic, which inhibits the growth of the two red algae, is secreted into the culture medium by the *Ralfsia* thalli. Certainly, reports on the production of antibiotics in this genus are not new: Conover and Sieburth²⁶ reported that tannins secreted from *Ralfsia verrucosa* (Aresch.) J. Ag. in tidepools may be suppressing barnacle and mussel colonisation.

The possible involvement of antibiotics as controlling factors in crustose algal formations adds a new dimension to existing interpretations based predominantly on differential growth rates and/or selective grazer activity. It also bears important implications for the growth of erect thalloid species, the success of which will depend on the establishment and sustained growth of the basal attachment systems.

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Possible location of chlorophyll within chloroplast membranes

THE inner membranes of the chloroplasts of higher plants and green algae contain the photosynthetic apparatus for the conversion of solar energy to chemical energy¹. Light is absorbed primarily by the chlorophylls, most of which act in a light-collecting capacity to absorb and transfer energy to the reaction centre chlorophylls of the two photosystems where the primary quantum conversion takes place². The chlorophylls comprise about 10% of the mass of mature chloroplast membranes or 20% of the total lipid mass. Because of their key role in photosynthesis, a question of prime importance is the location of the chlorophylls in chloroplast membranes. Earlier proposals have been reviewed by Kreutz³.

A plausible model for membrane structure is the fluid lipid-protein mosaic where the membrane continuum consists of a lipid bilayer in which intrinsic proteins are embedded and to which extrinsic proteins are attached. Although intrinsic proteins may be highly hydrophobic they are amphipathic molecules with a hydrophilic region located at the membrane surface; in some cases the molecule may extend through the membrane and have two hydrophilic regions, one at each surface of the membrane⁴⁻⁷. There also seem to be two classes of lipids: fluid, mobile lipids which make up the matrix of the lipid domain; and fixed, boundary lipids which consist of monomolecular layers attached to the hydrophobic shells of the penetrating intrinsic proteins^{8,9}.

Assuming the lipid-protein mosaic model to be valid for chloroplast membranes, the following reasons suggest that the bulk of the amphipathic chlorophyll molecules would be boundary lipids. If chlorophylls were located in the fluid lipid domain, the molecules would be free to move laterally in a random fashion through the membrane and the specific arrangement of the porphyrin rings essential for energy transfer would be impossible. This difficulty would not arise if the porphyrins were located in the protein region where ordered arrangement would be feasible. Indeed, it is likely that chlorophyll is attached to protein because two chlorophyll-protein complexes have been isolated from chloroplast membranes, following their solubilisation with sodium dodecyl sulphate. These two complexes make up some 70% of the intrinsic protein mass of mature chloroplast membranes: chlorophyll-protein complex 1, associated with photosystem 1, comprising some 20% and chlorophyll-protein complex 2, associated with photosystem 2, some 50% of the intrinsic protein^{10,11}. Moreover, it seems likely that most of the chlorophyll is attached to these complexes *in vivo*.

As well as porphyrin-protein interaction, the phytol chains would be associated with the hydrophobic regions of the two major intrinsic proteins. In a detailed molecular model proposed for chloroplast membranes, Weier and Benson¹² placed phytol chains, together with the acyl chains of the other chloroplast membrane lipids, into the hydrophobic interiors of lipoprotein subunits. Although this model¹³, in which the membrane is composed entirely of a sheet of lipoprotein subunits, is no longer considered feasible^{4,14}, it is possible to consider that the phytol chains will reside exclusively within the hydrophobic regions of the intrinsic proteins of the lipid-protein mosaic membrane. Alternatively, I propose that phytol could be associated with the hydrophobic exterior of the intrinsic proteins and thus chlorophyll would be part of the boundary lipids of the two chlorophyll-protein complexes. Less energy would be required to attach the phytol chains to the protein exterior than to bury them completely in the hydrophobic protein

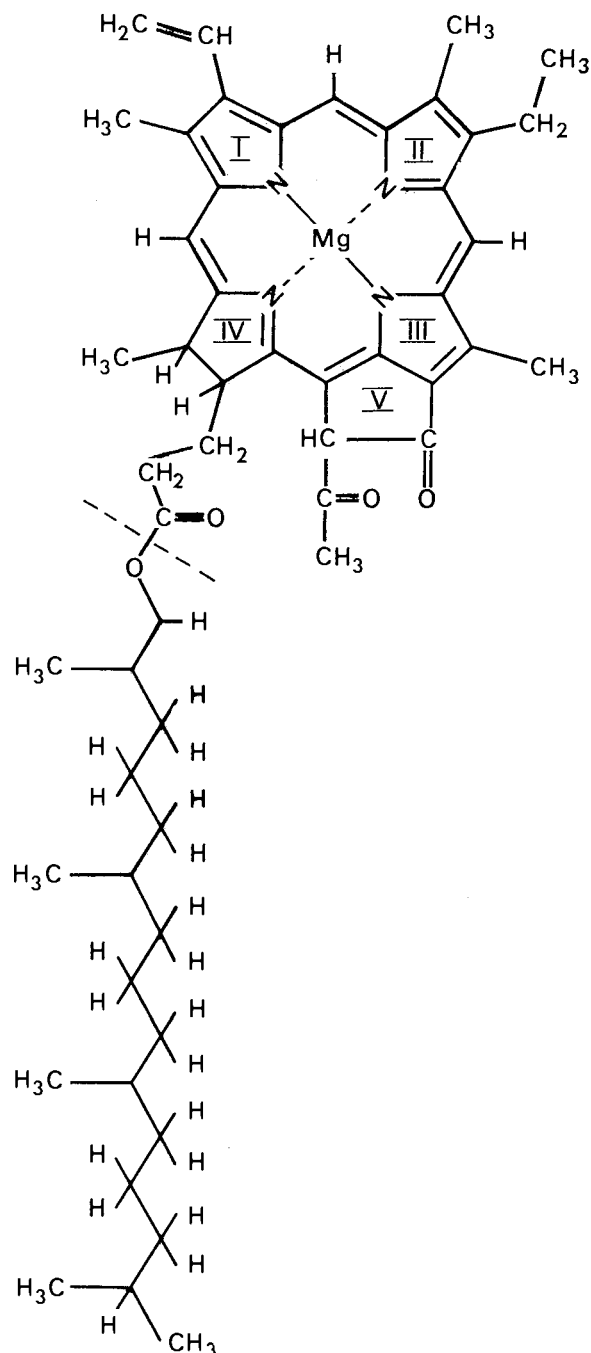


Fig. 1 Chlorophyll α .

interior. Further, anchoring the phytol chains at the protein exterior ensures that they will be perpendicular to the plane of the membrane and allows the porphyrin rings to be tilted with respect to the membrane surface.

Physical measurements indicate that the porphyrins are orientated at an angle of 48° to the membrane surface¹⁵. This orientation is analogous to that observed when chlorophyll is artificially incorporated into bilayers¹⁶⁻¹⁸. One edge of the square planar tetrapyrrole macrocycle, adjacent to phytol, is hydrophilic since it includes the cyclopentanone ring V and the carbonyl group of the propionic side chain of ring IV, to which phytol is attached; the Mg^{2+} also has hydrophilic properties (Fig. 1). This hydrophilic edge would interact at the aqueous membrane surface with the hydrophilic region of the intrinsic protein giving the porphyrin

ring the required tilt; the more hydrophobic portion of porphyrin would thus extend further into the hydrophobic protein region of the membrane. A schematic diagram is shown in Fig. 2 with chlorophyll molecules surrounding an intrinsic protein; additional lipids would be required to complete the boundary lipid monolayer. The necessary but undefined aggregations of the porphyrins would be possible in this model, and it would be expected in such arrays that the chlorophyll molecules will occur in several environments arising from interactions with other chlorophyll molecules, thus giving rise to the complexity of the *in vivo* spectra^{19,20}.

An additional reason for placing the light-harvesting chlorophylls as boundary lipids is evident when one considers the lipid content of chloroplast membranes. If intrinsic proteins are to be covered with boundary lipids as much as 35% of the total lipid molecules of chloroplast membranes may need to be immobilised. At least 30% of the total lipid mass consists of functional lipids, such as the chlorophylls, carotenoids and plastoquinones, which are not involved in formation of the bilayer. Obviously it is advantageous to use the chlorophylls, and probably also some carotenoids, as boundary lipids in order to leave sufficient lipids to form the fluid matrix.

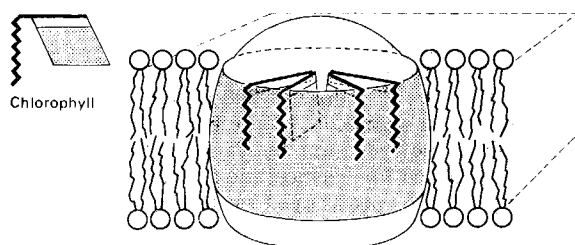


Fig. 2 Schematic cross section of a chloroplast membrane showing an intrinsic protein spanning the membrane, with hydrophilic regions located at the membrane surfaces and a hydrophobic portion (shaded) embedded within the nonpolar interior of the lipid bilayer. The chlorophyll molecules (shown on the left, with the hydrophobic portion of the porphyrin ring shaded) are part of the boundary lipid of a chlorophyll-protein complex. The phytol chains are perpendicular to the membrane surface in close interaction with the outside perimeter of the hydrophobic region of the intrinsic protein, and thus in contact on one side with protein and on the other side, with the acyl chains of the fluid lipid matrix. In contrast, the porphyrin rings are buried within the protein; the hydrophilic edge of the porphyrin ring is located at the membrane surface and the hydrophobic part is buried within the hydrophobic interior of the intrinsic complex.

If the chlorophylls are boundary lipids of the two major chloroplast intrinsic proteins as postulated, the porphyrins would be located towards the external or internal surface of the membrane. It is unlikely that the chlorophylls of an individual pigment complex would be divided between the outer and inner surfaces since this would markedly decrease the efficiency of light trapping. Briantais *et al.*²¹ favour the view that the porphyrin rings of the two photosystems are located on opposite sides of the membrane, with photosystem I at the external surface. On the other hand, Radunz²², using an antiserum monospecific to chlorophyll α , showed that part of the chlorophyll α of both photosystems was accessible to antibodies. Thus, a definitive answer to the question is not yet available. An important consequence, however, of chlorophyll being a boundary lipid is that the chlorophyll molecules will not be uniformly distributed over the membrane; rather they will occur only where the intrinsic proteins are located.

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Prostaglandins as haemostatic agents

It has been reported that prostaglandin endoperoxides (PGG₂ and PGH₂), possible intermediates in the biosynthesis of PGE₂ and PGF_{2α} from arachidonic acid, may be mediators of platelet aggregation¹⁻³. Kloeze reported⁴ that neither PGE₂ nor PGF_{2α} cause platelet aggregation or ADP release, and Willis⁵ stated that prostaglandins themselves cannot induce platelet aggregation. But we have found that three synthetic prostaglandins, Wy-16, 991, Wy-17, 185 and Wy-17, 186, induce platelet aggregation *in vitro*, and two of them, Wy-17, 185 and Wy-17, 186, significantly shorten the Lee White clotting time in rats. The most active of the three, Wy-17, 186, exerts a haemostatic effect on a bleeding wound surface.

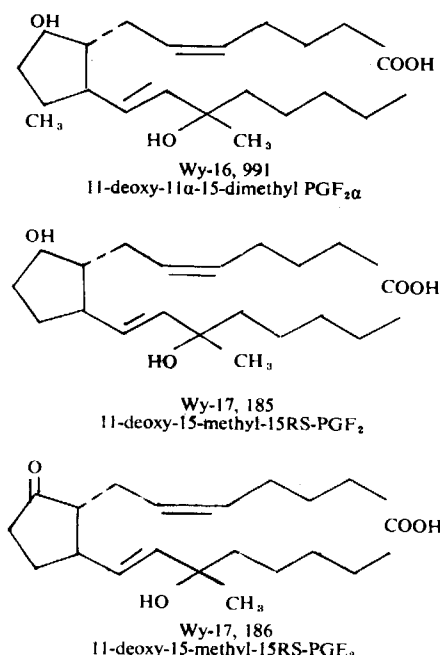


Table 1 Effect of prostaglandins on Lee White clotting time of fasted male rats

Compound	Dose ($\mu\text{g kg}^{-1}$)	No. of rats	Mean Lee White clotting time ($\pm$ standard error)		5 min
			1 min	No. of rats	
Control	—	8	341.2 $\pm$ 13.1	7	340.7 $\pm$ 13.6
Wy-17, 186	250	11	330.0 $\pm$ 22.9	9	296.7 $\pm$ 18.5
			NS		NS
Control	—	9	378.3 $\pm$ 12.4	8	356.3 $\pm$ 25.1
Wy-17, 186	500	9	281.7 $\pm$ 12.4	7	242.1 $\pm$ 24.9
			$P < 0.01$		$P < 0.01$
Control	—	9	393 $\pm$ 10.9	8	401 $\pm$ 8.4
Wy-17, 18	500	11	358 $\pm$ 10.9	8	363 $\pm$ 9.0
			$P < 0.05$		$P < 0.05$
Control	—	10	405.0 $\pm$ 21.1	8	373.1 $\pm$ 24.9
Wy-17, 185	250	10	363.8 $\pm$ 14.2	5	369.0 $\pm$ 22.2
			NS		NS
Control	—	8	433.1 $\pm$ 9.8	5	435.0 $\pm$ 9.2
Wy-16, 991	500	8	405.0 $\pm$ 12.5	6	412.5 $\pm$ 16.3
			NS		NS

Human blood was collected in siliconised 50 ml Vacutainers (Becton and Dickinson) fitted with siliconised 18 gauge needles using 3.8% sodium citrate as the anticoagulant (9 parts of blood to 1 part of sodium citrate). Platelet-rich plasma (PRP) was separated from the red blood cells and the effect of the prostaglandins on platelet aggregation was studied with a Payton Aggregation Module as described previously⁶.

To test the effect of these compounds on blood coagulability, groups of ether-anaesthetised male rats were given intravenous injections of the prostaglandin in physiological saline or saline control solution. Blood was obtained by cardiac puncture for the determination of the Lee White whole blood clotting time either 1 or 5 min after injection.

The haemostatic activity at a bleeding wound surface was determined by either exposing the femoral vein of Nembutal-anaesthetised rats and making a standardised nick (1–2 mm) in the vein with a lancet, to yield a free flow of blood, or opening the abdominal cavity to expose the mesenteric matrix of small blood vessels and making a 1–2-cm cut across these vessels with a scalpel. A 200 or 400 $\mu\text{g ml}^{-1}$ solution of the prostaglandin in physiological saline, or plain saline, was dripped on to the bleeding surface at a rate of about 1 drop per 5 s, and the time required for the cessation of bleeding was measured. The prostaglandins used were synthesised by Drs D. Strike and W. Kao.

A typical plot of the platelet aggregation induced in human PRP by these prostaglandins (3 $\mu\text{g ml}^{-1}$) compared with that induced by ADP (Fig. 1) shows them to be slightly less active

than ADP. Maximal platelet aggregating activities of Wy-17, 185 and Wy-17, 186 at 26 μM are inhibited 50% by 0.033 μM and 0.092 μM PGE_1 , respectively. It is interesting that the aggregating activity of these prostaglandins can be inhibited by another prostaglandin, PGE_1 .

Table 1 shows the effects of the prostaglandins on the Lee White clotting time. In a dose of 500 $\mu\text{g kg}^{-1}$ Wy-17, 185 and Wy-17, 186 had a significant effect both 1 and 5 min after injection. Since the reduction by Wy-17, 186 was somewhat greater, it was tested for haemostatic activity *in vivo*.

When Wy-17, 186 was dripped on to the wound surface of the femoral vein of 10 rats at a concentration of 200 $\mu\text{g ml}^{-1}$, the bleeding time was 77.8 $\pm$ 5.6 s compared with 97.4 $\pm$ 6.2 s for 10 controls. This bleeding time reduction was significant ($P < 0.05$). When a 400 $\mu\text{g ml}^{-1}$ concentration of Wy-17, 186 was instilled over the bleeding mesenteric vascular bed, the bleeding time for seven rats was 174 $\pm$ 16.9 s, compared with 395 $\pm$ 25 s for six controls. This reduction in bleeding time was significant ($P < 0.001$).

Apart from the endoperoxides, the activity of the stable prostaglandins reported here represents a unique example of prostaglandins capable of inducing platelet aggregation. Furthermore, Wy-17, 185 and Wy-17, 186 can shorten the Lee White clotting time, and Wy-17, 186 significantly shortens the bleeding time from a wound surface. The control of bleeding from a wound surface is a first step in the healing process. A potential application of the activity of this haemostatic prostaglandin may be in the prevention of excessive postsurgical bleeding, or in helping to control oozing of blood from a wound surface during surgery.

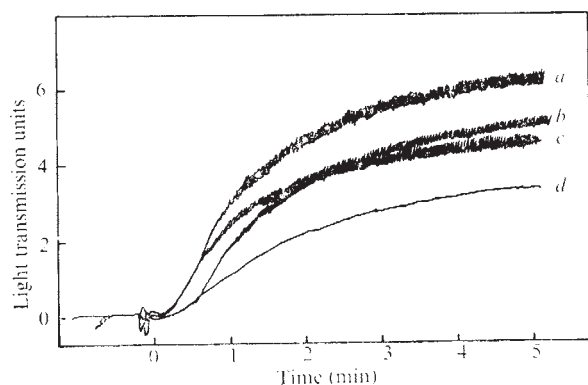
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Fig. 1 Effect of prostaglandins on platelet aggregation (a) 8.5 μM Wy-17, 185; (b) 8.5 μM Wy-17, 186; (c) 8.1 μM Wy-16, 991; (d) 3 μM ADP used as a comparative reference.



Effects of temperature and pressure on short term storage of platelets

NONE of the methods suggested so far to improve the storage life of platelets has proved entirely satisfactory¹⁻⁵. Storage at 22° C is the most accepted method³, and other suggestions include the use of chemical additives such as prostaglandin E₁ (ref. 5) or freezing techniques⁷⁻⁹. Studies involving a controlled rate of freezing and thawing in the presence of dimethyl sulphoxide have been encouraging¹⁰⁻¹², but a better method is desirable as the need for platelets is increasing rapidly. An improved method of storage might be developed if the normal discoid shape could be maintained. Furthermore, if the change in shape that occurs when platelets are cooled is accompanied by an increase in volume, as has been suggested¹³⁻¹⁵, or if the strength of the bonds maintaining the secondary and tertiary structure of the protein of the platelets is affected significantly by temperature or pressure, the application of sufficient pressure might shift the equilibrium towards a conformation favouring the discoid form. We have now found that pressure (2,500–9,000 pound inch⁻²) (17.24–61.65 N m⁻²) prevents to a considerable degree these changes in shape, and restores the shape of platelets in concentrates that have been kept at 2° C and atmospheric pressure for 1–2 h.

We first monitored the changes in shape using a water-jacketed high pressure optical cell in a Cary 14 spectrophotometer and samples of human and bovine blood platelet-rich plasma and platelet concentrate when pressure and temperature were varied. (Human platelet concentrates, from the Community Blood Bank of Kansas City, were prepared by collecting 500 ml of whole blood in a Fenwall GA-35 transfer pack containing 70 ml of acid citrate dextrose solution. Samples were centrifuged at 3,000g for 3 min, and the supernatant platelet-rich plasma was centrifuged at 5,000g for 5 min. The platelets were then resuspended in about 30 ml of plasma.) When low temperature studies were conducted a stream of nitrogen was passed through the cell compartment to prevent water condensation. Cooling consistently caused an increase in the absorbance which was reversed when pressure was applied to the cooled sample. A typical

Fig. 1 Absorbance at 620 nm of a platelet concentrate diluted 1:2 with saline solution as a function of applied pressure. ○, Absorbance values measured 5 min after application of the designated pressure; □, absorbance corrected for the effect of pressure at room temperature. The sample is ~24 h old.

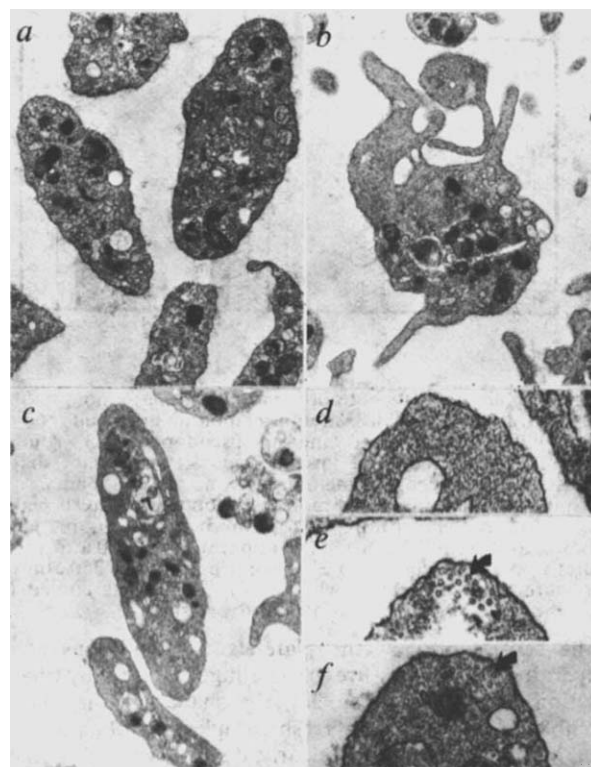
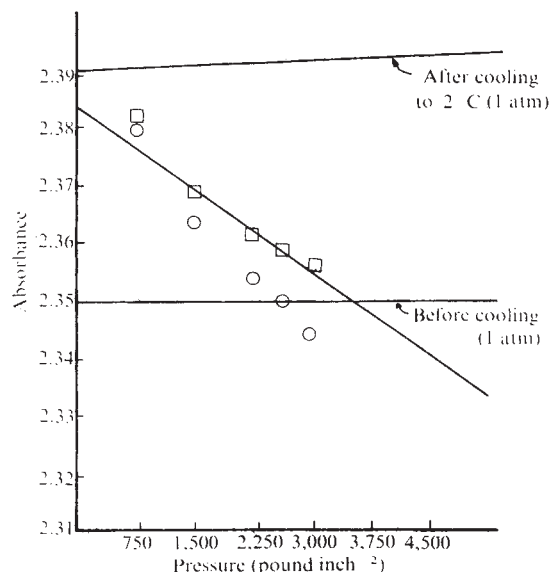


Fig. 2 Electron micrographs of platelets showing: *a*, typical discoid profile at room temperature and 1 atm of pressure; *b*, the numerous pseudopodial extensions and rounded form at low (2° C) temperature and 1 atm pressure, and *c*, discoidal forms at low temperature accompanied by pressure. (*a-c*, $\times 13,400$). Micrographs at higher magnification of the margin of platelets show (*d*) the absence of the marginal band of microtubules at low temperature with pressure, (*e*) the marginal band of microtubules (arrow) at room temperature and 1 atm pressure and (*f*) the band (arrow) at room temperature and 5,000 pounds inch⁻² (34.48 N m⁻²) pressure. (*d-f*, $\times 23,830$).

example of the data thus obtained is shown in Fig. 1. In several cases, pressure decreased absorbance to the original value (that is, the value at room temperature), but, in some cases, pressure seemed to cause only a slight decrease in absorbance. In the same apparatus, pressure followed by cooling caused a smaller increase in absorbance than did cooling alone.

For verification, and as an independent check on these results, we also used transmission electron microscopy. A high pressure cell with a dual chamber (a modified form of that described in ref. 16) was used to fix the platelets under high pressure at a controlled temperature. Suspensions of platelet concentrate were maintained in one of the chambers, and a solution of 5% glutaraldehyde with s-collidine buffer was kept in the other. When temperature, time and pressure were appropriate, the fixative was mixed with the platelet suspension to give chemical fixing in the desired conditions.

Figure 2 shows typical results obtained when a batch of platelet concentrate was examined under four conditions. At room temperature the platelets were discoid with profiles in thin sections ranging from circular to elongated ellipsoid. Figure 2*a* shows a typical elongated profile of a fresh platelet. Beneath the generally smooth contour of the surface membrane was a marginal band of microtubules circumscribing the uniformly distributed vesicles and granules in the cytoplasm (Fig. 2*e*). When cooled, virtually all platelets become rounded, with irregular surface contours associated with the presence of numerous fine pseudopodia (Fig. 2*b*). The marginal band of microtubules was lacking and the membranous organelles and granules were grouped together

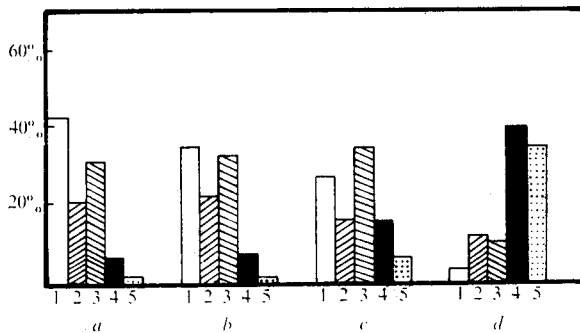


Fig. 3 Relative distribution of various shapes among platelets. 1, Definitely discoid, smooth membrane no pseudopodia; 2, round, smooth membrane no pseudopodia; 3, round, smooth membrane, one pseudopod; 4, irregular shape (caused by irregular membrane), no pseudopodia; 5, irregular shape and one or more pseudopodia; *a*, fresh platelets at room temperature and 1 atm pressure; *b*, platelets cooled to 2°C for 1 h after the application of 350 atm pressure; *c*, platelets cooled to 2°C for 1 h and then 350 atm of pressure applied for 1 h (while cool); *d*, platelets cooled to 2°C for 1 h at 1 atm pressure.

in the central area of the platelets. In conditions of low temperature with pressure or cooling followed by the application of pressure (Fig. 2c), the shapes of the platelets resembled those found in fresh samples at room temperature. Many platelets were clearly discoid with smooth surfaces and uniformly distributed organelles and granules. These discoid platelets had no marginal band of microtubules (Fig. 2d), although some were found in the central part of these platelets among the membranous organelles. Thus, microtubules do not seem to be essential to the maintenance of the discoidal shape of platelets under these conditions.

In an attempt to quantitate our results, we took photographs at low magnification and counted the platelets in each of five arbitrarily chosen categories based on shape and structure. The categories and their relative populations are presented in Fig. 3. About 93% of our fresh platelets fitted categories 1, 2 and 3, which were characteristic of normal fresh platelets. Treatment at 2°C for 1 h resulted in 25% platelets in categories 1, 2 and 3, and 75% in categories 4 and 5. More than 90% of the platelets cooled under pressure for 1 h fitted categories 1, 2 and 3, while 78% of those cooled for 1 h and then pressurised while cold for 1 h fitted categories 1, 2 and 3, with 22% in categories 4 and 5. Thus, it seems that pressure prevented the change in shape that typically occurs when platelets are cooled. Figure 3d indicates that pressure was less effective after cooling than before cooling.

In discoid cells maintained at low temperature under pressure there was no marginal band of microtubules (Fig. 2d) in contrast to platelets kept at room temperature and atmospheric pressure (Fig. 2c). To evaluate this further, platelets were fixed at room temperature under pressure, and examined with the electron microscope. In all cases, there was a marginal band of microtubules (Fig. 2f) indicating that, contrary to many suggestions¹⁷⁻²⁰ pressure alone in the ranges investigated did not cause disaggregation of the microtubules. Similar findings were obtained with microtubules from various nerve cells using the equipment described here²¹. The marginal band was generally not as clearly segregated into discrete bundles in these conditions as their counterparts at a pressure of 1 atm.

The fact that pressure definitely moderates the morphological changes caused by cooling, indicates that the use of physical methods (either alone or in conjunction with other methods) may lead to improved storage procedures for blood platelets. The measurement of some viability parameters of platelet samples subjected to various conditions of pressure and temperature is currently in progress.

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Mechanism for oscillatory synthesis of cyclic AMP in *Dictyostelium discoideum*

CELLS of the slime mould *Dictyostelium discoideum* aggregate around centres into multicellular fruiting bodies as a chemotactic response to cyclic AMP (refs 1 and 2). The wave-like character of this phenomenon suggests the release of cyclic AMP pulses by the founder cells^{3,4}. In this respect, metabolic oscillations controlled by cyclic AMP have been observed in suspension cultures of *D. discoideum* cells capable of forming aggregates⁵. We present here a mechanism for both these intracellular oscillations and the periodic release of chemotactic signal by aggregation centres.

The mechanism is based on the regulation of two enzymes directly involved in the synthesis of cyclic AMP, ATP pyrophosphohydrolase (E_1) and adenylyl cyclase (E_2), which transform ATP into 5' AMP and cyclic AMP, respectively⁶ (see Fig. 1). In *D. discoideum*, this bienzyme system bound to the cell membrane is autocatalytically controlled: adenylyl cyclase is activated by 5' AMP whereas ATP pyrophosphohydrolase is activated by cyclic AMP (ref. 6). In both cases positive heterotropic interactions are highly cooperative, suggesting the oligomeric structure of the enzymes. The system is further coupled to a membrane-bound phosphodiesterase which transforms cyclic AMP into 5' AMP (ref. 7). Rossomando and Sussman⁸ have suggested that the above regulatory pattern plays a primary role in the periodicity of aggregation. We support this hypothesis by a model which shows that the regulatory properties of adenylyl cyclase and ATP pyrophosphohydrolase can give rise to sustained oscillations in the synthesis of cyclic AMP around a non-equilibrium unstable stationary state.

The variables considered in the model are the intracellular concentrations of ATP, 5' AMP and cyclic AMP. The destruction of cyclic AMP in the phosphodiesterase reaction is represented, for the sake of simplicity, by a linear term corresponding to a non-saturated Michaelian enzyme. The system is open, ATP being produced at a constant rate and 5' AMP being removed in proportion to its concentration; the only sink taken into

account for cyclic AMP is that of the phosphodiesterase reaction. As this paper deals with the molecular basis of cyclic AMP oscillations at the cellular level and not with the process of slime mould aggregation, the effect of diffusion is not considered. It should be stressed, however, that oscillations in the intracellular cyclic AMP concentration may result, through transport of this metabolite across the cell membrane, in a periodic release of chemotactic factor into the extracellular medium. The cooperative kinetics of ATP pyrophosphohydrolase and adenylyl cyclase is described by the concerted model of Monod *et al.*⁸, assuming that these proteins are allosteric dimers and that the substrate and positive effectors bind exclusively to the active (R) state of each enzyme. In conditions of a quasi-stationary state for the enzymatic species, the time evolution of the metabolite concentrations is then given by the differential equations^{9,10}

$$\dot{\alpha} = v_1 - \frac{\epsilon_1 \alpha (1 + \alpha) (1 + \gamma)^2}{L_1 + (1 + \alpha)^2 (1 + \gamma)^2} - \frac{\epsilon_2 \alpha (1 + \alpha) (1 + \beta)^2}{L_2 + (1 + \alpha)^2 (1 + \beta)^2} \quad (1a)$$

$$\dot{\beta} = \frac{\epsilon_1 \alpha (1 + \alpha) (1 + \gamma)^2}{L_1 + (1 + \alpha)^2 (1 + \gamma)^2} - k_1 \beta + k_2 \gamma \quad (1b)$$

$$\dot{\gamma} = \frac{\epsilon_2 \alpha (1 + \alpha) (1 + \beta)^2}{L_2 + (1 + \alpha)^2 (1 + \beta)^2} - k_2 \gamma \quad (1c)$$

where α , β and γ denote, respectively, the normalised concentrations of ATP, 5' AMP and cyclic AMP; L_1 and L_2 , and ϵ_1 and ϵ_2 , are, respectively, the allosteric constants and the normalised maximum activities of enzymes E_1 and E_2 . The source term of the substrate is denoted v_1 , whereas k_1 and k_2 are the rate constants for the sink of the products β and γ . Concentrations and kinetic parameters (v_1 , ϵ_1 , ϵ_2) are normalised by division through the appropriate Michaelian or dissociation constants of enzyme-metabolite complexes^{9,10}.

The system described by equations (1a-c) admits a stationary state (α_0 , β_0 , γ_0) for $\dot{\alpha} = \dot{\beta} = \dot{\gamma} = 0$, that is, when the following relationships are satisfied:

$$\begin{aligned} \beta &= v_1/k_1 \\ \gamma &= [\epsilon_2 \alpha (1 + \alpha) (1 + \beta)^2] / k_2 [L_2 + (1 + \alpha)^2 (1 + \beta)^2] \\ [\epsilon_1 \alpha (1 + \alpha) (1 + \gamma)^2] / [L_1 + (1 + \alpha)^2 (1 + \gamma)^2] &= v_1 - k_2 \gamma \end{aligned} \quad (2)$$

The dynamic behaviour of the model depends on the stability properties of the stationary state (α_0 , β_0 , γ_0). The latter have been investigated using a normal mode analysis¹¹ after graphical resolution of equations (2) on a digital computer. The results of the stability analysis are indicated in Fig. 2 as a function of

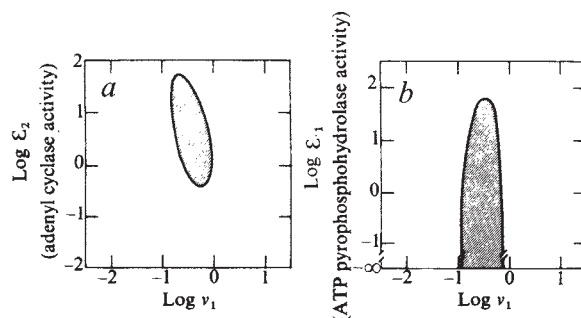


Fig. 2 Domain of sustained oscillations (shaded area) as a function of ATP injection rate (v_1), adenylyl cyclase and ATP pyrophosphohydrolase activity. The diagrams are established for $L_1 = L_2 = 10^6$, $k_1 = k_2 = 0.1 \text{ s}^{-1}$. a, $\epsilon_1 = 10 \text{ s}^{-1}$; b, $\epsilon_2 = 10 \text{ s}^{-1}$. Outside the shaded area the system evolves towards a steady state corresponding to a constant intracellular cyclic AMP level and, in the case of a linear sink to the extracellular medium, to steady release of chemotactic signal.

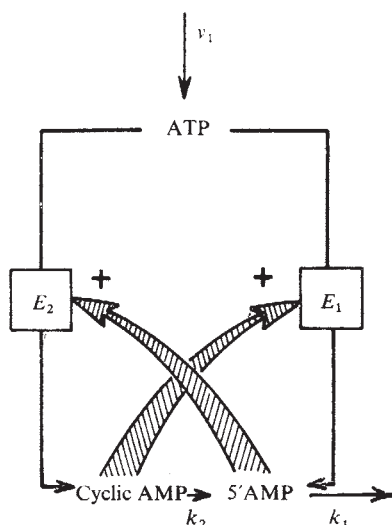
adenylyl cyclase and ATP pyrophosphohydrolase activity, for varying ATP injection rate. Each diagram is divided into two domains: outside the dashed region, the system evolves toward a stable steady state, whereas in the shaded domain of temporal dissipative structures^{11,12}, the system undergoes sustained oscillations around a non-equilibrium unstable stationary state with unique amplitude and frequency (Fig. 3). The period of the phenomenon is extremely stable with regard to enzymatic activities and substrate injection rate; it varies by less than a factor of two in the oscillatory range of parameter v_1 and a similar variation obtains for ϵ_1 and ϵ_2 . The oscillatory domain of each enzyme activity extends over more than two orders of magnitude, in contrast to the narrow unstable range observed for the substrate input (Fig. 2). Increasing ϵ_1 , ϵ_2 or v_1 lowers the period, whereas the amplitude of the oscillations passes through a maximum with respect to v_1 and ϵ_2 and diminishes with increasing ϵ_1 from zero. The role of enzyme cooperativity in the mechanism of instability is illustrated by the fact that the oscillatory domain corresponds to large values for the allosteric constants L_1 and L_2 , of the order of 10^6 . Predictions as to the order of actual metabolite concentrations in the course of oscillations are beyond the scope of the present study and require a precise knowledge of *in situ* kinetic parameters of membrane enzymes E_1 , E_2 .

Despite its relative simplicity, the model yields a qualitative agreement with a number of experimental facts. The waveform of the oscillations in Fig. 3 accounts well for the pulsatory production of cyclic AMP observed in slime mould aggregation; on the other hand, the restricted range of the period corresponds quantitatively to the observation that founder cells in *D. discoideum* oscillate with a period of 3–5 min⁴. Both the pulsatory nature of the oscillations and the stability of the period result from the allosteric properties of the enzymes involved in the instability mechanism, as previously shown^{10,13} for phosphofructokinase in the case of glycolytic oscillations¹⁴.

The model also accounts for some of the experiments performed in cell suspensions. It should be stressed that these experiments⁵ suggest the existence of a metabolic oscillator controlled by cyclic AMP and the independent operation of an amplification mechanism, whose nature is still not clear, for the relay of cyclic AMP pulses^{3,4}. The latter process, linked to the membrane receptor for cyclic AMP, allows control of intracellular cyclic AMP synthesis by extracellular cyclic AMP. The response coupled to the cyclic AMP receptor is not considered in the model of Fig. 1, which relates only to the multi-enzyme oscillator, but provision is made for the model oscillatory system to react to externally added cyclic AMP as described below.

The interaction of cyclic AMP with the oscillating system has been studied experimentally in suspended cells by investigating the response to pulses or continuous injection of this metabolite.

Fig. 1 Mechanism for the oscillatory synthesis of cyclic AMP in *D. discoideum*, based on the activation of ATP pyrophosphohydrolase (E_1) by cyclic AMP and of adenylyl cyclase (E_2) by 5' AMP. Constants k_1 and k_2 relate to the 5' nucleotidase⁶ and phosphodiesterase reactions (see text).



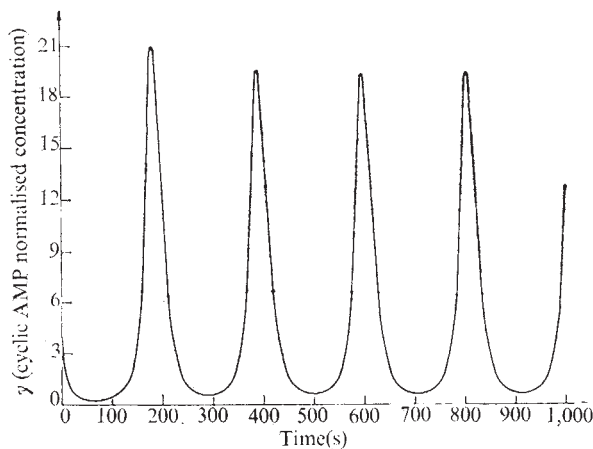


Fig. 3 Sustained oscillations of cyclic AMP. The curve is obtained by integration of equations (1a-c) using the Continuous System Modelling Program on an IBM 370-165 computer, for the following set of arbitrary values of the parameters: $\epsilon_1 = 0.4 \text{ s}^{-1}$, $\epsilon_2 = 10 \text{ s}^{-1}$, $k_1 = K_2 = 0.1 \text{ s}^{-1}$, $v_1 = 0.5 \text{ s}^{-1}$, $L_1 = L_2 = 10^6$. Initial conditions: $\alpha = 8$, $B = 4$, $\gamma = 4$. The normalised concentrations of ATP (α) and 5' AMP (β), (not shown) oscillate in the range 15-67 and 0.6-18, respectively. Periodic variation of intracellular cyclic AMP may result in a periodic release of chemotactic factor as a result of transport across the cell membrane.

Continuous addition of cyclic AMP does not modify the period of the phenomenon until injection rates are reached which extinguish the oscillations⁵. Correspondingly, in the model, addition of a constant source term in the kinetic equation (1c) for γ changes the period by less than 5% below the value 0.1 s^{-1} in the case considered in Fig. 3; above this threshold, oscillations disappear as the system evolves towards a steady state. As to the effect of discontinuous cyclic AMP addition, computer simulations of the model indicate that pulses of γ in the first part of the period after a peak of cyclic AMP delay the oscillations, with a maximum effect near the middle of the period; a small advance in the phase is observed when the pulse is applied in the second part of the period. These results and the fact that the minimum phase shift is found in the vicinity of a peak agree with experimental observations⁵. The differential sensitivity of the adenyl cyclase oscillator to cyclic AMP suggests a molecular basis for the refractory period observed in the chemotactic signalling process during aggregation³⁻⁵.

Two further remarks can be made about the experiments in suspended cells of *Dictyostelium*. The fact that 5' AMP does not interact with periodic behaviour⁵, contrary to the predictions of the model, could result from the lack of a membrane receptor for this metabolite. On the other hand, the model suggests that oscillations observed in the cytochrome chain⁵ result from the periodic variation of ATP in the adenyl cyclase system.

As a result of the formation of 5' AMP from cyclic AMP in the phosphodiesterase reaction, oscillations still arise in the absence of ATP pyrophosphohydrolase as indicated in Fig. 2. Thus periodic behaviour may result from the regulatory properties of adenyl cyclase only, although the reverse is not true for the second enzyme. For $\epsilon_1 = 0$, the model yields a three-variable autocatalytic mechanism for glycolytic oscillations with activation of phosphofructokinase by AMP; α , β , and γ denote then the concentrations of ATP, AMP and ADP, respectively. This generalises the results previously obtained in a two-variable model¹⁰ for glycolytic oscillations and stresses the similarity between the oscillatory mechanism in glycolysis and cyclic AMP synthesis. No interference between these oscillatory phenomena can be expected in aggregating cells of *D. discoideum* since glycolytic periodicities in yeast and muscle are linked to the cooperative properties of phosphofructokinase^{10,11}, which is not allosteric in the slime mould¹⁵.

Non-oscillatory mutants of *D. discoideum*, such as *ap66* (ref. 4), and oscillatory mutants with altered periodic properties,

such as *Fr17* (ref. 16), presumably differ from the wild type in the regulatory properties of one of the enzymes involved in the unstable mechanism¹⁷. More specifically, the model suggests that non-oscillatory mutants may lack activation of adenyl cyclase by 5' AMP. Particularly relevant is the finding that the regulatory pattern observed in *D. discoideum* (Fig. 1) also obtains in *Polysphondylium violaceum*⁶. Oscillations with a period of 1.5 min have been observed during aggregation in this species¹⁸, suggesting that the chemotactic attractant in *P. violaceum* is a metabolite linked to the oscillating system.

At the supracellular level, some models for *D. discoideum* aggregation postulate the existence of an oscillatory source of cyclic AMP (ref. 19). The mechanism discussed here lends support to such a postulate. Moreover, equations 1 a, b and c could be included, with the effect of diffusion, in a set of equations that describe the aggregating system²⁰. The model should then be extended to include a second mechanism for the relay of cyclic AMP pulses, linked to the membrane receptor for this metabolite. Wave-like aggregation should result.

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Ion movement across leukocyte plasma membrane and excitation of their metabolism

A PERTURBATION of the molecular organisation of the surface membrane of mammalian phagocytes leads to significant modifications of their metabolism and functions^{1,2}. These modifications include a rapid increase of oxygen uptake, of glucose oxidation by the hexose monophosphate pathway (HMP) and of hydrogen peroxide production¹⁻³; an enhanced turnover of membrane phospholipids⁴ and a degranulation with extrusion of lysosomal proteins from cells^{5,6}. The importance of this functional response of phagocytes is particularly evident when the alteration of their surface properties is caused by interaction with bacteria, which, on being engulfed, can be killed and digested by the toxic agents (for example, H_2O_2 , superoxide anions, cationic proteins and hydrolytic enzymes) produced and/or discharged into the phagocytic vacuole⁶⁻⁹.

The trigger of the metabolic excitation, which has puzzled several investigators in the past, has so far escaped identification. We have shown^{1,2} that concanavalin A (con A), both in solution and coupled to non-phagocytosable beads, induces a metabolic stimulation of leukocytes, which can be reversed by dislodging the cross-linking lectin from its membrane binding sites with appropriate sugars. We have suggested that the 'on-off' switch, operating at the level

of the leukocyte surface membrane, involves a process of association-dissociation of membrane protein subunits. The clustering of these subunits should lead to a local increase in membrane potential and/or permeability to ions^{1,10,11}. To test this hypothesis we have now studied the effect of lanthanum ion and some ionophores on the metabolism of polymorphonuclear leukocytes (PMNL). The rare earth cation has been shown to bind to cell membrane protein and to induce an ion redistribution across the membrane¹². The ionophores easily form complexes with alkali or alkali earth ions. The complexes freely diffuse across membranes, thereby mediating a passive transport of cations down their concentration gradient¹³.

The ionophores used were valinomycin and nigericin, which form lipid soluble complexes with monovalent cations^{13,14}; A23187, a carboxylic antibiotic which transfers Ca^{2+} and Mg^{2+} across biological membranes¹⁵; and X537A, another carboxylic antibiotic which binds and equilibrates both monovalent and divalent cations¹³. PMNL were isolated from peritoneal exudates of guinea pigs injected with sterile 1.2% caseinate 15–16 h earlier. Cells were suspended in either calcium-free Krebs-Ringer phosphate with 1.2 mM Mg^{2+} (KRP-Mg) or calcium-free Krebs-Ringer Tris with or without 1.2 mM Mg^{2+} (KRT-Mg and KRT, respectively). Tris buffer was used instead of phosphate to avoid formation of precipitates with lanthanum and calcium ions. Respiration of PMNL was monitored by means of a Clark-type oxygen electrode and glucose oxidation by counting $^{14}\text{CO}_2$ produced from labelled glucose¹. Stock solutions of the ionophores were prepared in dimethyl sulphoxide (DMSO), the final concentration of which in the cell suspensions never exceeded 0.5% (v/v).

About 2 min after the addition of lanthanum ions to PMNL suspended in KRT-Mg, there was a pronounced increase in cell respiration (Fig. 1). At the same time, the leukocytes underwent an activation of glucose oxidation which was measurable even at 10^{-5} M concentrations of La^{3+} (Fig. 2). The use of exogenous glucose labelled either in position 1 or 6 led to the conclusion that the only metabolic pathway activated was the HMP.

Also the addition of X537A to PMNL caused a very rapid and extensive enhancement in oxygen uptake (Fig. 1). Because of the response time of the oxygen probe used (90% in 10 s) and the time required to inject the ionophore into the cell suspension, we conclude that the onset of metabolic activation is almost immediate. On a concentra-

tion basis the stimulatory efficiency of X537A in KRP-Mg is far greater than that of valinomycin, nigericin or A23187. The stimulation of respiration by all these agents is not affected by 1 mM NaN_3 , thus ruling out an activation of the mitochondrial oxidative systems. In addition, the stimulation of oxidation of 1- ^{14}C -glucose, and not of 6- ^{14}C -glucose (Table 1), shows that the respiratory stimulation is linked to an increase in the rate of HMP.

As well as acting as ion carriers, the ionophores might exert their biological effects by a mechanism unrelated to the ion movement. At least for A23187 this possibility seems unlikely, in view of the results obtained in media containing different electrolytes. As shown in Fig. 3, when A23187 is added to PMNL suspended in KRT, a slight stimulation of respiration is observed. This activation is very likely due to calcium bound to leukocytes or present as a contaminant in the reagents used. In fact, if Ca^{2+} is added to the cell suspension before A23187, a much greater increase in the rate of oxygen uptake is obtained. A similar potentiation of the effect of the ionophore is also given by Mg^{2+} , which, on a concentration basis, is less active, however, than Ca^{2+} . This difference might be explained as the result of either the steeper inward gradient of Ca^{2+} concentration, or a higher sensitivity of the cytoplasmic target to Ca^{2+} than Mg^{2+} . A third possible explanation would be that high concentrations of magnesium ions displace Ca^{2+} from cell membrane binding sites, thereby making it available for complexing and transport by A23187.

Table 1 Effect of ionophores on ^{14}C -glucose oxidation by PMNL

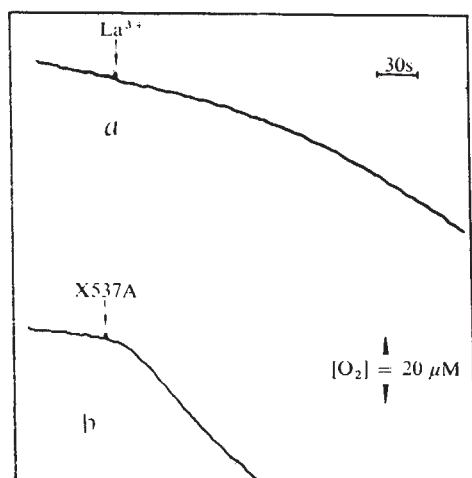
	Rest (DMSO)	X537A	A23187	Valinomycin
1- ^{14}C -glucose	16,075	61,150	33,475	30,975
6- ^{14}C -glucose	115	110	90	90

Data are expressed as $^{14}\text{CO}_2$ c.p.m. per μCi ^{14}C -glucose per 5 min per 1×10^7 cells. DMSO, 0.25%; ionophores, 20 μM . For experimental details see Fig. 2.

The activity of the divalent cations might be linked to an exchange with alkali ions. A23187 catalyses a K^+ release from erythrocytes¹⁵ and the parotid gland¹⁶, which is greatly stimulated by calcium ions. A similar migration of cations might also take place in leukocytes. Indeed, valinomycin and nigericin, which equilibrate K^+ concentrations across various membranes, cause a moderate metabolic stimulation of leukocytes. The activity of valinomycin cannot be amplified by an uncoupler such as 2,4-dinitrophenol, which enhances K^+ efflux from erythrocytes¹⁴, or by inhibitors of ATPase such as oligomycin or ouabain. Further, there is no synergy between valinomycin and A23187. Thus, while a divalent cation⁹ is an essential element in eliciting the metabolic response, the question whether or not K^+ participates in the process still remains open. It is important to emphasise, however, that X537A, the only ionophore among those used which is capable of carrying both alkali and alkali earth ions, provides the most efficient stimulus. Further, La^{3+} , which is also a good stimulant, has been shown both to deplete Ehrlich ascites tumour cells of K^+ (ref. 12) and displace Ca^{2+} from various biological membranes¹⁷.

Changes in the intracellular concentration of calcium ions seem to control the activity of various secretory cells. For example, A23187 and X537A cause a release of histamine from mast cells¹⁸, by promoting Ca^{2+} influx and extrusion of secretory granules¹⁹. Woodin has shown that the exocytosis of granule proteins from leukocytes treated with leukocytin is accompanied by loss of potassium and accumulation of calcium ions²⁰. It would be expected that at least some of the agents we have used are able to promote

Fig. 1 Stimulation of the rate of respiration of guinea pig polymorphonuclear leukocytes by La^{3+} or X537A. 2×10^7 cells were suspended in, *a*, 2 ml calcium-free Krebs-Ringer Tris buffer (KRT-Mg) or, *b*, 2 ml calcium-free Krebs-Ringer phosphate buffer (KRP-Mg), containing 0.2 mM glucose (37°C). The oxygen uptake was continuously recorded with a Clark-type electrode. LaCl_3 , 0.3 mM; X537A, 20 μM .



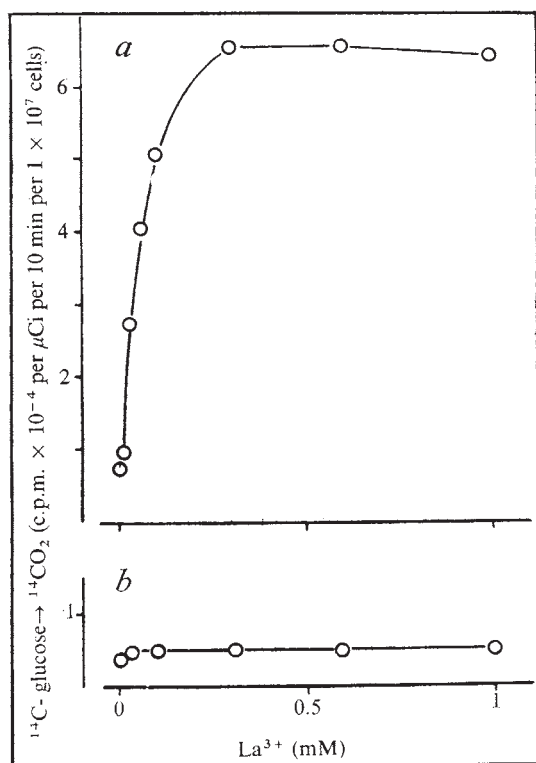
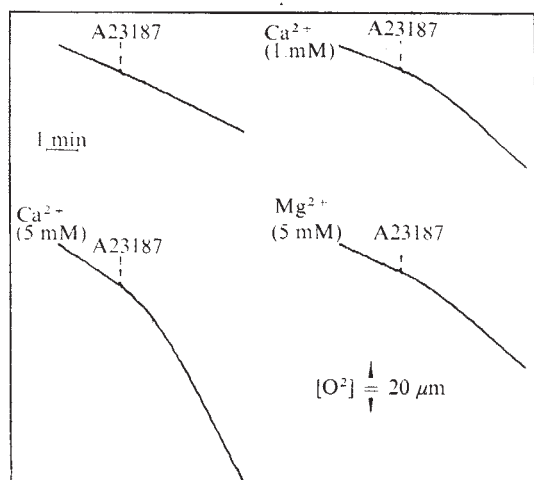


Fig. 2 Effect of lanthanum ions on the rate of ^{14}C -glucose oxidation by guinea pig polymorphonuclear leukocytes. 1×10^7 cells were incubated at 37°C in 2 ml of KRT-Mg, containing 0.2 mM glucose, in rubber-capped flasks. Different amounts of LaCl_3 were then added, followed by, a, $0.4 \mu\text{Ci}$ of $1\text{-}^{14}\text{C}$ -glucose or, b, $4 \mu\text{Ci}$ of $6\text{-}^{14}\text{C}$ -glucose. After 10 min, the reaction was stopped with $1 \text{ N H}_2\text{SO}_4$; $^{14}\text{CO}_2$ was collected in 20% KOH, present in the flask centre well, and counted by scintillation.

a release of lysosomal enzymes from PMNL. Preliminary experiments have shown that X537A causes a moderate exocytosis of β -glucuronidase from PMNL. The appearance of the lysosomal enzyme in the medium is associated, however, with a parallel leakage of cytoplasmic enzymes such as lactate dehydrogenase and glucose-6-phosphate dehydrogenase. As reported by Goldstein *et al.*²¹, A23187 exhibits a very slight potentiation of the release of β -glucuronidase from human PMNL in the presence of Ca^{2+} . Thus, the relationship between the stimulation of the PMNL oxidative metabolism by ionophores and the selective release of lysosomal proteins remains to be clarified.

Fig. 3 Effect of added Ca^{2+} and Mg^{2+} on stimulation of guinea pig leukocytes by A23187. 2×10^7 cells suspended in 2 ml of KRT, containing 0.2 mM glucose. A23187, $10 \mu\text{M}$.



In conclusion, all the results presented here may be used to build a model of metabolic excitation of leukocytes. It is tempting to hypothesise a single, unique mechanism responsible for all the effects evoked in PMNL either by phagocytosable matter or by other surface perturbing agents (anti-PMNL antibodies, phospholipase C, detergents, fatty acids, con A and so on)². The trigger mechanism would consist, in fact, in a rapid shift of divalent cations from the cell environment and/or from the plasma membrane binding sites towards special zones of the cytoplasm. Calcium ions are likely candidates for this task. Other ion species, possibly K^+ , would be released in the opposite direction at the same time. According to this picture the leukocyte excitation would follow a mechanism rather common to other excitable cells.

Admittedly, this hypothesis requires further experimentation. Measures of actual fluxes of ions, ion gradients and dependence of the metabolic effects on these parameters are now under way in our laboratory.

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T-cell populations with different functions

VARIOUS functions have been ascribed to thymus-derived (T) cells, including the ability to synergise with B cells in antibody responses¹ and with T cells in the generation of effector functions (refs 2–4 and H. Cantor and E. Simpson, unpublished), cytotoxic activity⁵, and regulator or suppressor activity^{6,7}. T-cell subpopulations, defined by criteria such as quantity of cell surface θ antigen, homing characteristics (H. Cantor and E. Simpson, unpublished) and sensitivity to the effects of adult thymectomy and anti-thymocyte serum^{2,8}, have been described, but the functions of given subpopulations are still unclear. It seems likely, by analogy with B-cell differentiation and commitment to antibody class, that T cells become committed to certain functions during differentiation. In the absence of markers for individual T cells and their products or functions, analysis of cell populations and their activities provide

Table 1 CBA/J spleen cells sensitised to DBA/2 antigens: development of cells with helper, cytotoxic and nonspecific suppressor activity

CBA/J* spleen cell transferred	Cytotoxic activity† against:		Anti-DNP antibody response of recipients boosted with (ABC × 10 ⁻⁸ M)	
	P815	EL-4	DNP-P815§	DNP-EL-4§
0	—	—	0.49 (0.37–0.64)	—
50 × 10 ⁶ normal	0	0	0.32 (0.23–0.45)	0.07 (0.04–0.12)
50 × 10 ⁶ grafted with DBA/2 skin, day 28	0	0	19.9 (14.1–25.8)	—
50 × 10 ⁶ grafted with DBA/2 skin, day 13	0	0	24.3 (15.4–38.3)	0.14 (0.09–0.22)
50 × 10 ⁶ cultured with irradiated DBA/2 spleen cells‡	19.90 ± 0.49	5.36 ± 0.17	0.14 (0.09–0.21)	—
50 × 10 ⁶ grafted day 13 plus 15 × 10 ⁶ cultured	—	—	2.35 (2.22–2.48)	—
50 × 10 ⁶ OVA primed	DNP _g -OVA 84.8 (68.7–105)			
50 × 10 ⁶ OVA primed plus 15 × 10 ⁶ cultured	8.53 (6.43–11.3)			

* CBA/J spleen cells were sensitised to alloantigens either *in vivo* by grafting tail skin or *in vitro* by the Wunderlich and Canty¹² modification of the Mishell-Dutton culture system¹³. Some mice were primed with 100 µg alum adsorbed ovalbumin (OVA) plus pertussis. Recipients were 400 r X-irradiated CBA/J mice. All received, in addition to the helper cells shown in the first column, 40 × 10⁶ spleen cells from CBA/J mice primed with 100 µg alum-adsorbed DNP-KLH plus pertussis 4 weeks previously and treated with 0.3 ml rabbit anti-mouse thymocyte serum (ATS) intraperitoneally 48 h before transfer as a source of B-cell precursors. Boosting was with 2 × 10⁷ DNP-P815 or DNP-EL-4, or with 5 µg DNP-OVA given intraperitoneally. Mice were bled 10 d later and anti-DNP antibody was measured by a modified Farr technique¹⁴. Geometric mean antigen binding capacity (ABC) for groups of five mice was determined; the value in parentheses is the range enclosed by one standard error.

† Cytotoxicity = % specific lysis
 $\frac{^{51}\text{Cr released by sensitised cells} - ^{51}\text{Cr released by non-sensitised cells}}{^{51}\text{Cr released from } 4 \times \text{frozen and thawed targets}} \times 100$

Titration of cytotoxic activity was performed according to the method of Canty and Wunderlich¹⁵ using a fixed number (5 × 10⁵) of ⁵¹Cr-labelled target cells and different numbers of attacking cells, usually 4 × 10⁶, 2 × 10⁶, 1 × 10⁶ and 0.5 × 10⁶ in 1-ml volumes using four replicates for each attacker: target cell ratio, that is attacker:target cell ratios of 8:1, 4:1, 2:1 and 1:1. Target cells were from the ascites tumours EL-4 (H-2^b) and P815 (H-2^d). Under the conditions used, cytotoxic activity was directly proportional to the attacker:target cell ratio. In this table, the figures for cytotoxic activity shown are for an attacker:target cell ratio of 8:1. Background spontaneous ⁵¹Cr release for P815 was 6.75% and for EL-4 was 4.35% (4-h assay).

‡ *In vitro* sensitisation: 20 × 10⁶ CBA/J spleen cells were cultured with 1 × 10⁶ 2500 r X-irradiated DBA/2N spleen cells in 1 ml volumes and collected after 5 d. Numbers of cells used in transfer refers to number of viable cells transferred.

§ P815 and EL-4 tumour cells were reacted with 1-fluoro-2, 4-dinitrobenzene in isotonic carbonate-bicarbonate buffered saline, pH 9.3, at room temperature for 15 min and washed thoroughly to yield DNP-P815 and DNP-EL-4.

an approach to this question. Experiments of this type have dissociated helper and cytotoxic activity^{9,10}, and the 'unmasking' of nonspecific suppressor T cells was reported to follow the culture of helper cells with specific antigen¹¹. We have now dissociated helper activity and cytotoxic activity directed towards a given alloantigen following either *in vitro* or *in vivo* immunisation. We also found that nonspecific suppressor T cells arise during *in vitro* culture.

To investigate whether cytotoxic T-cell activity and helper T-cell activity specific for the same alloantigens occurred in the same population of sensitised T cells, CBA/J mice were sensitised to DBA/2 and their spleen cells were assayed simultaneously for cytotoxic and helper activity. In the experiment illustrated in Table 1, spleen cells from CBA/J mice immunised *in vivo* by skin grafting 13 or 28 d previously had no cytotoxic activity but did demonstrate substantial helper activity (lines 3 and 4 compared with lines 1 and 2). In other experiments, weak, variable cytotoxic activity was observed in spleen cells of skin grafted mice; this activity did not correlate with helper activity. *In vitro* sensitised spleen cells, however, had considerable cytotoxic activity (the small amount of activity against EL-4 was probably the result of H-2 determinants shared by H-2^b and H-2^d, but not H-2^k mice); such cells had no helper activity (line 5). To test for suppressor activity, small numbers of *in vitro* sensitised cells were mixed with active helper spleen cells from mice immunised *in vivo* before transfer. With 15 × 10⁶ *in vitro* sensitised cells the anti-DNP antibody response was 10% of the expected value (line 4 compared with line 6). The suppressor activity was apparently nonspecific, for 15 × 10⁶ *in vitro* sensitised cells had the same effect on the anti-DNP antibody response to DNP_g-OVA in recipients of OVA-primed helper cells (lines 7 and 8).

Further experiments to test the specificity of helper and cytotoxic cell activity and of the suppressive effect are shown in Table 2. In this experiment, spleen cells from CBA/J mice immunised *in vivo* with either DBA/2 or C57BL/6 antigens were transferred to irradiated recipients together with DNP-primed spleen cells and the appropriate antigen. *In vivo* immunised cells had helper activity specific for the priming alloantigen but

Table 2 Generation of suppressor cells in the absence of killer cells

CBA/J spleen cells*	Cytotoxic activity† against:		Anti-DNP antibody response of recipients boosted with (ABC × 10 ⁻⁸ M)	
	P815	EL-4	DNP-P815	DNP-EL-4
50 × 10 ⁶ normal	0	0	0.26 (0.18–0.36)	0.05 (0.03–0.10)
50 × 10 ⁶ grafted with DBA/2 skin, day 15	0	0	3.8 (3.0–4.8)	0.32 (0.20–0.51)
50 × 10 ⁶ grafted with C57BL/6 skin, day 15	0	0.60 ± 0.16	0.46 (0.31–0.67)	1.48 (0.73–3.00)
50 × 10 ⁶ grafted plus 20 × 10 ⁶ cultured with irradiated DBA/2 spleen cells‡	3.00 ± 0.14	0.72 ± 0.21	1.31§ (0.87–1.97)	0.64 (0.38–1.06)
50 × 10 ⁶ grafted plus 20 × 10 ⁶ cultured with irradiated C57BL/6 spleen cells‡	0	37.6 ± 0.19	1.77 (1.32–2.38)	0.51 (0.28–0.94)
50 × 10 ⁶ grafted plus 20 × 10 ⁶ cultured with irradiated CBA/J spleen cells‡	0	0	0.94§ (0.70–1.26)	0.18§ (0.11–0.27)

* DNP-B cell precursor donors treated with ATS as in Table 1.

† Cytotoxic activity, as in Table 1. Figures shown are for attacker:target cell ratios of 8:1. P815 background ⁵¹Cr release was 7.07%, EL-4, 5.87% (4-h assay).

‡ Cytotoxic data for these groups is for the cultured cells alone. After transfer of spleen cells from grafted donors, recipients were boosted with DNP on the homologous tumour cell, that is DNP-P815 for recipients of DBA/2 skin grafted donor spleen cells, DNP-EL-4 for recipients of C57BL/6 skin grafted donor spleen cells.

§ Suppression of anti-DNP response significant at *P* < 0.05 by two-tailed Student's *t* test.

Table 3 Effect of anti- θ serum and complement on cytotoxic and suppressor cells

CBA spleen* helper cells transferred	CBA cells tested for cytotoxic and suppressor activity	Cytotoxic activity† against P815	Anti-DNP antibody response of recipients boosted with DNP-OVA (ABC $\times 10^{-8}$ M)
50 $\times 10^6$ normal	—	—	0.1
50 $\times 10^6$ OVA primed	—	—	21.5 (18.4–25.3)
50 $\times 10^6$ OVA primed	15 $\times 10^6$ normal spleen	0	32.8 (25.5–42.1)
50 $\times 10^6$ OVA primed	15 $\times 10^6$ cultured with irradiated DBA/2 spleen cells	25.6 $\pm$ 0.4	7.4 (5.9–9.3)
50 $\times 10^6$ OVA primed	15 $\times 10^6$ cultured with irradiated DBA/2 and treated with anti- θ and C'‡ following collection at day 5	7.2 $\pm$ 0.3	18.5 (16.6–20.7)

*Cell transfers and cultures as in previous Tables.

†Cytotoxic activity as in Tables 1 and 2. Figures given are for attacker: target cell ratio of 4:1; background ^{51}Cr release for P815 was 7.97% (4-h assay).

‡Anti- θ serum; conventional AKR anti- θ C3H used at 1 ml of a 1:4 dilution per 10^8 cells; C', guinea pig complement.

little or no cytotoxic activity (lines 2 and 3). When *in vitro* sensitised cells which had specific cytotoxic activity directed against the sensitising antigen were mixed with the *in vivo* primed helper cells before transfer, all anti-DNP antibody responses decreased (lines 4 and 5), and even greater suppressive activity was given by CBA/J spleen cells held in culture for 5 d with irradiated syngeneic cells (line 6). The suppressor activity was therefore essentially nonspecific both in induction and expression.

Finally, Table 3 shows that suppressor cells in the cultured spleen cell population are T cells. CBA/J spleen cells sensitised *in vitro* to DBA/2 antigens and showing cytotoxic activity against H-2^d target cells were tested as nonspecific suppressors *in vivo* by mixing them with DNP-KLH-primed and OVA-primed CBA/J spleen cells. The anti-DNP antibody response of the recipients to DNP-OVA was suppressed by the cultured cells (line 4 compared with lines 2 and 3) but this effect was abrogated by treatment of the cultured cells with anti- θ plus complement, which also substantially reduced their cytotoxic activity. This strongly suggests that the suppressive effect is a T-cell function.

Helper activity and cytotoxic activity are both T-cell functions and demonstrate specificity for antigen. The activities are dissociated in these experiments since immunisation regimes which favour the production of one apparently induce little of the other. The suppressor activity present in those populations with high levels of cytotoxicity could, however, mask the activity of helper cells induced in the same populations. While helper and cytotoxic activities are not manifest in the same populations and are probably functions of different T-cell subpopulations, the present evidence is not decisive. Nor do these data bear on the question of whether the cells with these effector activities are derived from the same or different precursor cells. Data obtained with anti-Ly antisera before and after sensitisation suggest, however, that helper cells and cytotoxic cells occur in different populations and are derived from different precursors (P. Beverly and H. Cantor, personal communications).

The suppressor activity we have described is also a T-cell function but differs from helper and cytotoxic activity in being nonspecific both in induction and expression. The suppressor cell generated by 4–5 d of culture under Mishell–Dutton conditions is interesting for several reasons. While Kontiainen and Feldmann¹⁶ have reported the generation of specific T helper cells in Marbrook cultures, helper cell generation in Mishell–Dutton cultures has not been observed. This failure may well be due to masking of helper cells by suppressor cells. Indeed, Kontiainen and Feldmann (personal communication) found that helper activity was optimal at 3–4 d of culture and declined thereafter, perhaps due to generation of suppressor cells. Furthermore, *in vitro* primary antibody responses tend to 'turn off' after 5–6 d even when the medium is changed regularly. This phenomenon may well result from supervening suppressor activity of the type we describe. Direct evidence that this

may be occurring has recently been provided by experiments in which the *in vitro* anti-DNP response to DNP-Ficoll, a T-independent antigen¹⁷, was prolonged solely by pretreatment of the cultured cells with anti- θ and C' (D. Mosier, personal communication). The suppressor cell, described by Pierce¹⁸ and Dutton¹⁹, that arises in spleen cells cultured for 2 d with concanavalin A may be the same type of cell; the concanavalin A might serve primarily to accelerate its activation. The stimulus for the generation of the nonspecific suppressor T cells observed in our experiments is not known, as alloantigens are not required. The foetal calf sera used in these experiments, however, while from several different batches, all contained those unknown features which make for good *in vitro* immune responses (R. I. Mishell, personal communication). Such materials have mitogenic potential and this may account for the generation of suppressive activity. In parallel experiments the T-cell nature and lack of specificity of this suppressor cell have been confirmed, using an *in vitro* assay for its activity. Furthermore, it has been shown to suppress the *in vitro* antibody response to a T-independent antigen, suggesting that it may act directly upon B cells (P. C. Marrack and J. W. Kappler, unpublished).

In conclusion, T cells with three distinct activities have been demonstrated and dissociated: antigen specific helper T cells, antigen specific cytotoxic T effector cells and nonspecific T suppressor cells. Our data do not show whether these populations come from a common precursor nor, if so, at what stage they differentiated into distinct sublines of cells.

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Prolonged survival of muscle allografts in adult rats

ALLOGRAFTS from minced muscle tissue and free muscle grafts are retained, as are other types of allografts, if immunological tolerance is induced in the recipients by neonatal injection of antigen¹, but are rejected in untreated animals^{1,2}. Recovery of contractile and histochemical properties of the regenerating muscle fibres in such free muscle allografts in tolerant rats has been observed. This study was designed to determine the fate of free muscle grafts transferred to animals treated in adult life.

Rats of the AVN strain were immunised at the age of 2.5 months with antigen prepared from rats of the Lewis (Lew) strain, differing from the AVN recipients at the strong (H-1) and weak (non-H-1) histocompatibility loci. When the hydrocortisone injections were stopped, free grafts of the whole extensor digitorum longus (EDL) muscle were transplanted into the bed of the previously removed EDL muscle of the recipient. The tendon of the muscle was sutured to that of the removed muscle. The grafted fast EDL muscle undergoes a process of degeneration succeeded by regeneration during which it changes from the originally slow to a fast contracting muscle, and histochemically from a homogeneous to a mixed muscle fibre pattern. A detailed study of the changes in contractile and histochemical properties of such free grafts has been reported⁴. The recovery of grafted muscle may be complete with respect to twitch characteristics⁵ but rather incomplete with respect to weight and tetanic tension output and morphological changes⁴⁻⁷.

One group of AVN rats received transplanted muscles from Lew donors (H-1 and non-H-1 difference) and a second group from the congenic strain Lew.1A donors, differing from the AVN recipients only at the weak (non-H-1) histocompatibility locus⁸. Thirty days after transplantation and combined treatment, the EDL muscles were removed and their contractile properties studied *in vitro*. The muscles were set up in a chamber with platinum electrodes for massive stimulation⁹ at a temperature of 37° C and the contraction properties recorded using an automatic analyser¹⁰. Twitch tension, latency period,

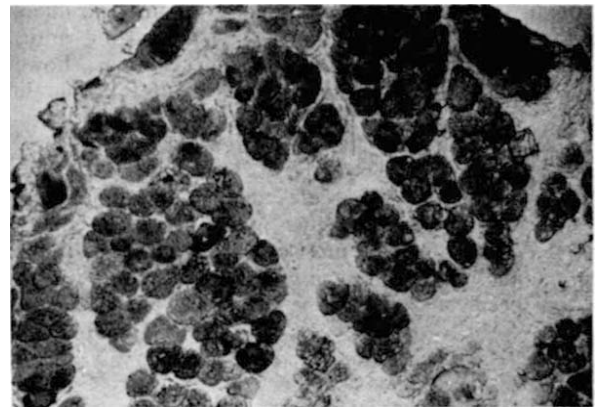


Fig. 1 Cross section through EDL muscle allograft (Lew.1A) in treated animal (AVN) 30 d after transplantation, stained for succinic dehydrogenase activity.

contraction time (time to peak), half relaxation time and maximal tetanic tension output were recorded. The muscle regenerates were removed whole and frozen quickly in CO₂; transverse sections were cut using a cryostat at 20° C and stained for succinic dehydrogenase activity, adenosine triphosphatase activity at pH 9.5 and phosphorylase activity. In some cases, routine histological observations after formalin fixation, paraffin embedding and staining with haematoxylin and eosin, were also made.

Table 1 shows the results obtained 30 days after grafting. Regeneration and concomitant recovery of contraction properties of both types of grafted muscles were observed. There was no clear difference, however, between the two groups ($n = 4$ in each group), that is between those with the weak antigenic difference (non-H-1) and those with the strong antigenic difference (H-1). The pooled data of both groups ($n = 8$) do not therefore differ from those of the individual groups. Recovery of contractile properties is somewhat less effective than that in autografts or allografts in tolerant rats¹, the contraction times being slower and the maximal tetanic tension output being slightly smaller.

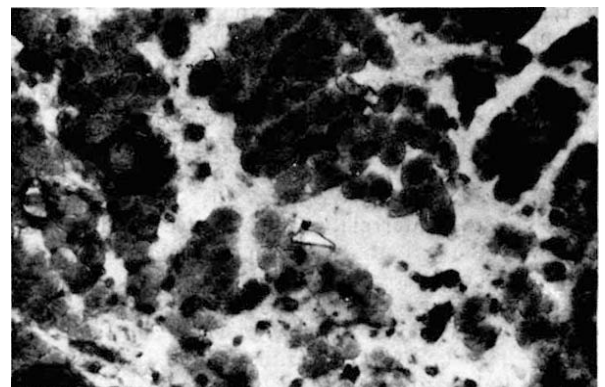
Figures 1 and 2 show the regenerating muscle fibres in cross section, stained for succinic dehydrogenase (Fig. 1) and adenosine triphosphatase (Fig. 2) activity. Untreated allografts are completely rejected (six out of six), the muscles being replaced by connective tissues and fat as described previously^{1,2}. Thus, pretreatment has a considerable effect on muscle allograft survival, although absolute survival time was not investigated. In preliminary experiments, to elucidate the mechanism of allograft survival, the serum collected from the treated animals, 45 days after muscle transplantation was transferred

Table 1 Contractile properties of free muscle allografts in treated animals

Type of graft	TT (g)	LP (ms)	CT (ms)	HRT (ms)	Tet.T. (g)	Weight of muscle (mg)
Lew.1A ($n = 4$)	2.70 ± 0.63	2.95 ± 0.22	17.95 ± 2.01	22.50 ± 2.01	10.70 ± 2.86	47.05 ± 9.24
Lew ($n = 4$)	1.46 ± 0.37	2.70 ± 0.30	17.38 ± 1.93	22.60 ± 2.93	6.03 ± 2.11	46.50 ± 15.05
Lew.1A + Lew	2.08 ± 0.41	2.82 ± 0.18	17.66 ± 1.29	22.55 ± 1.82	8.36 ± 1.87	47.00 ± 8.18

Treatment comprised immunisation of the recipients with donor antigen together with injections of hydrocortisone. Lyophilised spleen cells in saline were used as antigen and injected in fifteen doses five times per week, the first three doses being 3, 2 and 1 mg, respectively, and the subsequent doses, 0.8 mg. Hydrocortisone (Spofa, Prague) was injected in eight doses of 6.25 mg, each dose in 0.5 ml of distilled water. The first three injections were given on consecutive days and the subsequent five injections on alternate days³. Free allograft of the extensor digitorum longus muscle of weak (Lew.1A) or strong (Lew) antigenic difference transplanted into treated (hydrocortisone and injection of lyophilised spleen cells) rats (AVN). Data are given as mean $\pm$ s.e. Third row indicates results of both groups. TT, twitch tension; LP, latency period; CT, contraction time (time to peak); HRT, half relaxation time; Tet.T., maximal tetanic tension output.

Fig. 2 Cross section through EDL muscle allograft (Lew.1A) in treated animal (AVN) 30 d after transplantation, stained for adenosine triphosphatase activity.



in amounts of 4 ml per animal. The serum had no effect on allograft survival in the animals with the strong antigenic difference, so far tested. Allograft survival was, however, observed in some cases of animals with weak antigenic differences.

Recently, a case of a successful free autograft of skeletal muscle in man has been described¹¹. The possibility of muscle grafts in tolerant rats¹ and of inducing immunological enhancement of muscle allografts by the treatment described suggests the possibility of muscle grafting in man. Immunological enhancement of muscle grafts by the passive administration of specific antisera will be the subject of further studies.

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Repair of potentially lethal radiation damage in mammalian cells is associated with enhancement of malignant transformation

THERE have been several attempts to define conditions necessary for the fixation of the transformed state in mammalian cells *in vitro*. The relationship between fixation and proliferation after treatment has been studied in cell cultures exposed to methylcholanthrene¹, X irradiation^{2,3} and SV40 virus⁴. Cells were exposed to the carcinogen while in the density-inhibited plateau phase of growth, and subsequently subcultured at low cell density so that they could resume cell division. Transformation frequency was always reduced by delaying subculture for 24 h or longer, suggesting that cells must divide soon after exposure to a carcinogen in order to fix the transformed state. No data were reported for subculture less than 24 h after treatment.

Recent evidence, however, has shown that important repair processes, reflected by marked enhancement in survival when subculture is delayed after irradiation, may occur in density-inhibited cells during the first few hours after treatment^{5,6}. We have now studied the dynamics of transformation during this interval; changes in survival and transformation frequency were compared. We hope this approach may offer insight into the interrelationships between the repair of lesions with the potential of being lethal to the cell, and the development or loss of the potential of a cell to become transformed.

We used a C3H mouse embryo-derived cell line (10T-1/2 clone 8) provided by C. Reznikoff and C. Heidelberger (McArdle Laboratory for Cancer Research, University of

Wisconsin). This cell line has been adapted for use in quantitative transformation studies by Reznikoff *et al.*⁷ who found no evidence for spontaneous expression of murine C-type virus in these cells⁸. Stock and experimental cultures were maintained in Eagle's BME supplemented with 10% heat inactivated foetal calf serum (both obtained from Grand Island Biological Co). Stock and experimental cultures were maintained as outlined by Reznikoff *et al.*^{7,8}, except that cultures were kept in large glass stock bottles and the medium was supplemented with penicillin (50 U l⁻¹). Plateau phase experiments were performed with cells held as confluent monolayers for at least 24 h. The medium was changed 24 h before irradiation of the cells.

Transformation was scored as outlined before^{7,9}. The tumorigenicity of radiation-transformed foci was established by reinjection of cloned foci into syngeneic mice (Table 1). All tumours grew to 2–4 cm in diameter, when the mice either died or were killed. Type I foci, or dense regions of cells seen on experimental plates, were not scored as transformed and were not tumorigenic. Type II and III foci were tumorigenic and were scored as transformants. No spontaneous transformation was seen on untreated control plates. The morphology and corresponding tumorigenicity data found with radiation-transformed foci are consistent with those of Reznikoff *et al.*⁷ for methylcholanthrene.

Figure 1 shows results of experiments in which cells were irradiated in the density-inhibited plateau phase, and subse-

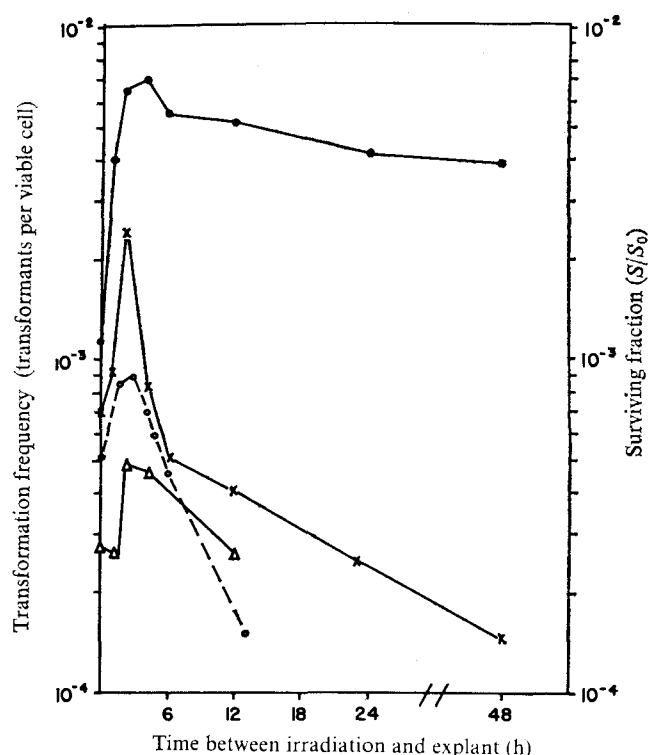


Fig. 1 Survival and transformation frequency in 10T-1/2 C3H mouse embryo cells irradiated in the density-inhibited plateau phase of growth and subcultured at various times thereafter. ●, Surviving fraction after 1,200 rad; △, transformation frequency after 200 rad; ○, 300 rad; ×, 400 rad. The confluent monolayers were trypsinised at various times (0–48 h) after irradiation, and subcultured at a lower cell density to assay for transformation and survival. Irradiation was carried out with a Phillips MG100 unit (100 kV, 10 mA) with 0.795 mm aluminium filtration and yielding a dose rate of 83.5 rad min⁻¹. For the transformation assay, cells were subcultured at a density such that approximately 200–500 viable cells per 100 mm Petri dish resulted, regardless of treatment. The medium was subsequently changed twice weekly until confluence was attained, then weekly until termination of the experiment 6 weeks after subculture. Dishes were then washed with normal saline, fixed with Bouin's solution, rinsed in 95% ethanol and stained with trypan blue. Lower density plates were also seeded at each point to assay for cell survival (plating efficiency) by standard techniques.

quently held in plateau phase for various times before subculture to assay for either survival or transformation. The upper curve, which represents cell survival, shows that survival was enhanced markedly when cells were held in plateau growth for 2–4 h after irradiation. The lower three curves represent the transformation frequency seen in similar experiments following 200, 300 and 400 rad irradiation. Transformation frequency was enhanced when cells were kept in plateau phase growth for 2–4 h after irradiation. The curves for survival and transformation were remarkably parallel for the earlier time intervals. With longer periods in plateau phase growth, however, a decline in the transformation frequency per surviving cell was consistently observed.

As Fig. 1 shows, there was an apparent increase in the amplitude of the transformation-enhancement with increasing radiation dose. A similar result has been described for the enhancement of survival, where the effect is seen as a decrease in the slope of the radiation survival curve⁶. A dose of 1,200 rad was thus chosen for the survival experiment, as the greater effect at higher doses allowed the kinetics of repair with time to be more easily studied. For transformation experiments, 200–400 rad were used as doses in this range yield maximum transformation per surviving cell⁹. The observed enhancement of survival would be fairly small following these lower doses.

These results demonstrate a high degree of correlation between the repair interval leading to both maximum survival and transformation following X irradiation. This observation sheds a somewhat different light on the relationship between delayed subculture and transformation in density-inhibited plateau phase cells as described before^{1–4}. A comparison of the data for subculture at 24–48 h after irradiation with those for 0 time shows a continual decline

a molecular level by other investigators^{10,11}. They report a peak in transformation frequency after treatment of synchronised cells with a radiomimetic alkylating agent at a time in the cell cycle when DNA strand breaks were being repaired at a maximal rate. The transformation frequency was maximal in cells treated during the 4-h interval preceding the G1/S boundary¹¹. Likewise, the repair of potentially lethal radiation damage also seems maximal in the late G1 cell population¹², the population of cells which predominates in plateau phase cultures. Taken together, these findings further support the error insertion hypothesis.

If the cell does insert the error which ultimately determines the behaviour of the cell, it may also be able to go back over its DNA and delete previously inserted errors. To be consistent with our results, this reversal of inserted errors would have to occur before the first cell division after treatment. It would thus be reflected by the decline in transformation frequency observed as the time in the density-inhibited plateau phase is lengthened. This hypothesis would also be consistent with the findings of Todaro and Green⁴ who treated cells with SV40 virus and then allowed one round of division before blocking them in a density-inhibited state. Under these conditions, no decline in the transformation frequency was observed.

Obviously, there is need for further investigation of the role of cell proliferation and molecular repair processes in oncogenic transformation; plateau cultures as a model for *in vivo* transformation may be useful. Our results indicate, however, that placing cells under conditions which enhance survival may be detrimental to the cells in terms of transformation. Furthermore, they suggest that if exposure to a carcinogen *in vivo* is associated with an appropriate proliferative response, slowly proliferating or non-actively-cycling cell populations, frequent targets of environmental carcinogens, may be particularly vulnerable to transformation.

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Table 1 Inoculation of C3H 10T-1/2 clone 8 normal and radiation transformed cells into syngeneic mice

Irradiation (rad)	Type of transformed colony	No. of cells injected per mouse	Tumour incidence (No. of tumours/No. of mice)
None	Normal cells	2–5 × 10 ⁶	0/4
600	III	2–4 × 10 ⁶	5/5
600	II	2–4 × 10 ⁶	3/4
600	I	2–10 × 10 ⁶	0/8
600	Normal (non-transformed)	2–10 × 10 ⁶	0/9

in the transformation frequency (Fig. 1). This finding is consistent with the observations with transformation induced by methylcholanthrene¹, radiation^{2,3} and SV40⁴, and with the hypothesis that if there is an interval after treatment during which a cell does not divide, it will repair the lesions or errors which would eventually lead to malignant transformation. The implication of this hypothesis is that the errors were produced as a consequence of the direct action of the carcinogen, probably on cellular DNA. Our data for repair intervals of less than 24 h suggest two alternative hypotheses: (1) errors may be inserted during the repair of radiation-induced lesions in the DNA; or (2) some severely damaged cells may repair sufficient damage to survive but these survivors retain an unusually high risk of transformation. To explain the results in Fig. 1 by the latter hypothesis, however, the transformation frequency per cell among this repaired population would have to be three to four times the maximum frequency ever observed in irradiated cells not allowed to repair. The insertion of new errors during repair would thus seem a more reasonable explanation for the findings.

The question of whether transformation involves direct induction of the critical lesion, or whether the error is inserted in the DNA during repair, has been dealt with on

Heterotransplantation of *Theileria parva*-infected cells to athymic mice

CONGENITALLY athymic (nude) mice have been used for the heterotransplantation and maintenance of human tumours^{1–6}. In many cases aplasia of the thymus is sufficient to allow tumour growth and other immunosuppressive treatments are apparently unnecessary. Here we describe how infected cells taken from a fatal case of East Coast fever

(ECF), regularly produced tumour-like masses only in irradiated athymic mice.

ECF is a tick-borne disease caused by *Theileria parva*, an obligatory intracellular protozoan parasite which undergoes schizogony in bovine lymphoid cells and which, in infected cattle, causes lymph node hyperplasia of almost lymphosarcomatous proportions⁷. It is at present the most important cattle disease in East Africa⁸.

An outbred colony of athymic mice was established from stock supplied 9 months before experimentation, from the MRC Laboratory Animal Centre, Carshalton, UK. Mice were maintained as an isolated colony under conventional conditions; those used for experiments were 6–8 weeks old and were irradiated at 800 rad from a ⁶⁰Co source 1 h before inoculation.

A steer was experimentally infected with ECF⁹ and died 20 days later. Immediately after death one iliac lymph node and one mediastinal lymph node were removed aseptically and finely chopped in sterile Eagle's MEM with added heparin (20 IU ml⁻¹). The cell suspension was pipetted off, centrifuged at 1,000g for 10 min and the cell pellet resuspended in Eagle's MEM, supplemented with 20% foetal calf serum, L-β-asparagine and antibiotics¹⁰. Lymphoid cells (5.6 × 10⁶) were thus obtained, of which 72.5% contained *T. parva* macroschizonts and 4.0% contained microschizonts. For mouse to mouse passage, tumour-like masses were similarly treated, but after removal were trypsinised for 4 × 30 min in 0.25% trypsin at 37° C, before pelleting and resuspending in Eagle's MEM. All inocula of mice were of 5.0 × 10⁷ cells in 0.2 ml of medium, given subcutaneously behind the right shoulder.

Cells obtained from the infected steer were inoculated into each of five irradiated athymic mice, and five irradiated Swiss mice. The residual cells were set up in culture¹⁰.

Between days 8 and 9 after inoculation, subcutaneous tumour-like masses were detected at the site of inoculation in four athymic mice. These continued to grow until the mice died on days 12, 33, 38 and 40. Biopsies of the masses showed the continuous presence of morphologically-normal parasitised bovine lymphoid cells. At *post mortem* examination, extensive vascular spread of these cells was seen on examination of blood and organ impression smears. Such cells, and occasional free macroschizonts, were particularly common in the lungs and liver, but were also seen in kidney, spleen, brain and lymph nodes. The tumour-like masses were composed almost entirely of parasitised bovine lymphoid cells and microschizonts were quite common in the later stages of growth. Piroplasms invading mouse erythrocytes were occasionally seen. Karyology of cells from a mass from one of the mice showed parasites present only in bovine cells. Despite the extensive vascular spread of parasitised cells, no tumour-like masses were detected in other sites on either macroscopic or histological examination. No macroscopic evidence of residual thymus tissue was detected *post mortem* in any of the mice examined.

No tumour-like masses developed in any of the Swiss mice, but lymphoid cells put into culture grew immediately.

The mass from the athymic mouse that died on day 33 was removed and a cell suspension prepared, aliquots of which were inoculated as before into five irradiated athymic mice. All five mice developed tumour-like masses, and passage was continued in irradiated athymic mice. Six passages were carried out and parasitised bovine lymphoid cells survived continuously in the mice for 136 d. During this time 47 irradiated athymic mice were inoculated and 43 developed parasitised tumour-like masses at the site of inoculation. Four mice died before growth was detectable.

The behaviour of cells in mice was similar in all passages and no evidence of further adaptation of the parasite was observed.

At passages 4 and 5 cells were also inoculated into a total of 8 non-irradiated athymic mice; two of these mice

developed small transient tumour-like masses and one developed a large persistent mass similar to those seen in irradiated mice.

Many athymic mice died as a result of the high dose of irradiation, but in some cases death was associated with massive infiltration of parasitised cells both locally and vascularity. In these cases respiratory distress, caused by pulmonary invasion by parasitised cells, was commonly seen as a prelude to, and as a probable cause of, death. The masses, which sometimes exceeded 20% of body weight, occasionally became excoriated on the surface and avascular necrosis was common in the larger ones. There was, however, no evidence of regression or rejection.

In Swiss mice, features associated with malignant neoplasia of lymphoid cells were previously recorded, following inoculation of *T. parva*-infected lymphoid cells grown in culture^{11,12}. Our findings support the concept of neoplastic-like growth of *T. parva*-infected cells in mice and provide a further step towards developing a laboratory model for use in ECF research, for example in chemotherapy, cross immunity studies and in the isolation of field infections. The invasion of mouse erythrocytes by piroplasms of *T. parva* has not previously been reported, although infected erythrocytes have been noted in irradiated athymic mice inoculated with *T. parva*-infected bovine lymphoid cells grown in culture (unpublished data). This finding opens up the possibility of interesting tick transmission studies.

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Transplantation and preliminary characterisation of lymphocyte surface markers of Abelson virus-induced lymphomas

MOST murine lymphomas induced by commonly available murine leukaemia viruses (such as Gross passage A and Moloney leukaemia viruses) arise in the thymus¹, originating as thymocytes or thymus-derived (T) cells. The tumours thus usually carry the surface θ antigen², they rarely

Table 1 Transplantation of Abelson virus-induced lymphosarcomas

Primary tumour No.	Days from virus injection to diagnosis of primary tumour	No. of primary cells injected i.p. $\times 10^5$	Frequency of ascites tumours in recipients		Time for development of ascites tumours in pristane-primed mice: mean and (range)	Times for development of non-ascites tumours in control mice: mean and (range)	
			Control	Pristane primed		Needle-track	Lymphoma
1	21	5	0/3	2/3	13(13)	19	43(43)
2	22	16	0/3	3/3	14(13-15)		44(36-60)
3	24	14	0/3	2/3	10(10)		55(51-57)
4	25	35	0/4	4/4	14(10-22)		47(36-50)
5	26	26	0/4	4/4	12(11-15)	18,18	42(35-49)
6	30	1	0/3	3/3	29(19-38)		43(38-45)
7	34	2.5	0/3	3/3	14(12-17)		39(34-41)
8	35	8.9	0/3	3/3	25(22-26)		39(33-51)
9	21	3.8	0/3	3/3	25(25)		48(45-53)
10	21	2.5	1*/3	3/3	9(8-10)	11*,15	32(32)

Abelson virus was obtained from Dr L. Rabstein, passaged in BALB/cAnN mice as 10% (w/v) tumour extracts. The pools used had a plaque-forming titre of 2×10^5 MLV PFU per 0.1 ml as measured by the XC test⁷ and a tumorigenic dose 50 (TD₅₀ of 2×10^2 per 0.1 ml when injected in newborn BALB/c mice). All pools also contained the LDH virus (which is not necessary for lymphoma induction by Abelson virus). Newborn mice were injected with 0.05 ml virus intraperitoneally (i.p.). Tumours arose in lymph nodes, meninges and bone marrow 3-5 weeks after injection (column 1). For transplantation, tumour cell suspensions were injected intraperitoneally into female BALB/c mice 2-4 months old, some pretreated 7-28 d previously with 0.5 ml pristane intraperitoneally (in transplantation of plasmacytomas, the pretreatment interval does not seem to be critical for transplantation enhancement). Ten primary tumours were used as the source of malignant cells to inject 32 pretreated mice and 32 controls.

* Same tumour.

have B-cell surface markers such as the κ (light) chain. Abelson and Rabstein, however, reported a leukaemia virus that induces non-thymic lymphomas³⁻⁵, which we have now found to have surface κ chains under certain circumstances.

When we started this work, transplantation of Abelson virus-induced tumours had not been reported. We therefore pretreated the recipients of the transplants with 0.5 ml of pristane intraperitoneally, as this had been previously shown to enhance the transplantation of plasma cell tumours⁶. As Table 1 shows, pristane-priming makes a striking difference in the efficiency of transplantation and the pathology of the transplanted tumour. Of the control mice, not treated with pristane, only 1 out of 32 developed an ascites tumour while four others developed local needle-track tumours (the control mouse with ascites also had a needle-track tumour). Local needle-track tumours presumably represent cellular transplantation rather than local virus induction of tumour. Of more than a thousand mice injected by us with Abelson virus, none has ever developed a needle-track tumour. The other 27 control mice developed a generalised non-thymic lymphoma after a mean latent period of 44 d (range 32-60 d). The gross distribution and microscopic pathology of the lymphomas arising in this group of mice was identical to that which results from inoculation of the cell-free virus into adult BALB/c mice. The pathology of these mice, infected with virus as adults, differs from that of those infected with virus as newborns by its longer latent period and the rarity of cranial bulging and facial tumours. Since the transplanted tumours all actively produce virus, the late-arising tumours in the non-pristane-treated mice very likely represent fresh tumour induction by virus. The later time of onset (32-60 d) of these tumours corresponds to those previously published for virus-induced disease in adult BALB/c mice⁸.

On the other hand, 30 out of 32 of the pristane-primed recipient mice developed ascites tumours, most of them very rapidly. It should be noted that seven out of the ten primary tumours transplanted successfully as ascites tumours with mean latent periods of 9-14 d. Successful transplantation of three of the ten primary tumours took longer, with means of 25-29 d. Since we have never obtained tumours in primed mice or unprimed mice by straight virus injection in less than 20 d we conclude that at least 7/10 primary tumours were successfully transplanted. More rigorous evidence that these tumours were indeed transplants rather than fresh virus inductions was provided by karyotyping four tumours which were transplanted into mice of the opposite sex (XX or XY karyotypes). Secondary

or later passages of tumour No. 10 and three others not listed in Table 1 were all the karyotype of the donor (primary) mouse. Thus, it seems that the pristane-primed mouse is a far superior recipient for the successful transplantation of this type of tumour.

One ascites tumour arising from the transplantation of each of the 10 primary tumours was studied for the presence of lymphocyte surface markers (Table 2). Each of the 10

Table 2 Surface markers on tumour cells

Tumour No.	K ⁺	Primary EA/EAC		Transplant	
		K ⁺	θ	K ⁺	EA/EAC
1	—	—	—	+	—
2	—	—	—	+	—
3	—	—	—	+	—
4	—	—	—	+	—
5	—	—	—	+	—
6	—	—	—	+	—
7	—	—	—	+	—
8	+	—	—	+	—
9	+	—	—	+	—
10	—	—	—	+	—
Others†	5/18†	0/3†	0/2†	22/23*†	

The gamma globulin fraction of a rabbit anti-mouse κ chain antiserum (a gift of Dr R. Mage) was conjugated with fluorescein isothiocyanate according to the method of Wood *et al.*⁹. Anti- θ C₃H was prepared according to the method of Reiff and Allen¹⁰. One-tenth millilitre of a cell suspension containing 5×10^6 tumour cells was incubated at 4°C with 0.1 ml of the fluoresceinated anti- κ reagent. The cells were then washed three times and one drop of the suspension was examined under ultraviolet light with a Leitz Ortholux microscope. If no immunoglobulin was detected with the direct technique by using the fluoresceinated anti- κ reagent, the tumours were then studied for the presence of the θ determinant by an indirect technique in which the cells were first incubated with the anti- θ diluted 1:10 as a middle layer followed by washing and then by the fluoresceinated anti- κ reagent. In this manner the fluoresceinated anti- κ reagent would bind to the κ chains of the mouse anti- θ antibody present on the θ -positive cells. For the detection of the C3 receptor of the B lymphocyte and the receptor for cytophilic antibody of the monocyte by red cell rosette techniques, the following reagents were prepared: 7SEA were prepared by adding the 7S rabbit anti-sheep cell antibodies (A) to sheep erythrocytes (E). 19SEAC were prepared by adding 19S rabbit antibody to E followed by an equal volume of fresh mouse serum (as the source of complement) diluted 1:10 in Veronal-buffered saline (VBS). These reagents were used as previously described¹¹. Rosette formation with the 19 SEAC reagent indicates the rosetted cells bear the C3 receptor of B lymphocytes; rosette formation with the 7S EA would indicate the cell had the cytophilic receptor of the monocyte. Under the conditions used the 7SEA reagent does not bind to the FC receptor of B cells¹⁰.

* One negative was a needle-track tumour.

† Abelson tumours not listed in Table 1.

transplanted ascites tumours listed in Table 1 as well as seven of the primaries and several other primary and transplanted tumours (shown as others on Table 2) was tested for the presence of surface κ chain. Seven tumours (Nos 1, 2, 5, 7 and three others) were also tested for their ability to form rosettes with EA or EAC. Six κ negative tumours (Nos 2, 4, 5, 7 and two others) were also tested for the presence of θ antigen. (The method used, indirect immunofluorescence, did not permit testing for θ in the presence of surface κ chain.)

The results of these tests were that all transplanted tumours had surface κ chain; 7 out of 25 primary tumours also had surface κ chain; 0 out of 6 tumours tested had θ antigen, and 0 out of 7 tumours tested formed rosettes with EA or EAC. These latter results indicated that the cells lacked the B-lymphocyte receptors for C3 and the monocyte receptor for cytophilic immunoglobulin.

The Abelson lymphomas are non-thymic in origin and, as demonstrated here, lack θ antigen; hence, they are unlikely to be derived from T lymphocytes. The basic question then is whether these cells are B-lymphocyte tumours, that is neoplastic derivatives of immunoglobulin-producing cells. The presence of κ chain on the surface of all the transplants and some of the primaries observed in this study suggests that this is indeed the case. It does not, however, prove that this surface immunoglobulin was synthesised by these cells. Another possibility is that the surface κ chains present on the tumours represent passively acquired host antibody directed against viral or tumour antigens on the surface of the neoplastic cells. While the data presented here do not rule out the latter possibility, we have in a separate report demonstrated IgM synthesis by long term tissue-culture sublines of tumour No. 5 (Table 1) (M.D.S., E. Premkumar, M.P. and P. Singer, manuscript in preparation). In addition, tumours No. 1 and No. 8 have also been shown to synthesise IgM (E. Premkumar, *et al.*, unpublished).

The results of this study together with the previous work⁸ on plasma cell tumour induction by Abelson virus indicate that this virus preferentially induces tumours in immunoglobulin-synthesising cells. The ability to obtain B-cell lymphomas rapidly, in great numbers, and perhaps with predetermined specificities, should be of great value to the cellular immunologist.

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Local anaesthetics increase susceptibility of untransformed cells to agglutination by concanavalin A

CELL agglutination by concanavalin A (con A) is accompanied by redistribution of con A receptors on the cell surface from a random pattern to form patches¹⁻⁵. Differences in the susceptibility of untransformed and transformed cells to agglutination by con A may therefore reflect differences in the ability of receptors to move laterally within the membrane to form patches. For example, it has been suggested⁶⁻⁸ that the higher susceptibility of transformed cells to agglutination by low doses of con A might be associated with a more fluid lipid plasma membrane matrix. Enhanced fluidity of the bilayer matrix would allow greater lateral movement of receptors within the membrane and favour formation of patches of receptors after binding of con A to the cell surface. The increased susceptibility of L cell nutritional auxotrophs to agglutination by con A after enrichment of their membranes with fluid phospholipids⁹ supports this view. We report here that local anaesthetics which increase the fluidity of phospholipids in model membranes also cause a significant increase in the susceptibility of untransformed cells to agglutination by low doses of con A.

BALB/c mouse 3T3 cells, 3T3 cells transformed by SV40 (SV3T3), BHK cells and BHK cells transformed by polyoma virus (PY-BHK) were grown in Dulbecco's modified Eagle's MEM in 50 mm plastic Petri dishes (Falcon) as before¹⁰. Preparation of affinity-chromatography-purified con A, measurement of ³H-con A binding to cells and quantitation of cell agglutination have also been described previously⁴. Cells for freeze fracture studies were suspended in a solution of glycerol (30% v/v), sucrose (0.25 M) and Tris (5 mM), pH 7.2, and centrifuged; cell pellets were frozen rapidly in Freon 22 and cleaved in a Balzers BA 360 freeze-etching apparatus and shadowed with platinum as before¹¹.

Maximum agglutination of untransformed 3T3 or BHK cells by con A required significantly larger doses of con A than their virus-transformed derivatives (Table 1). Incubation of 3T3 and BHK cells with the local anaesthetic dibucaine for 60 min at 37°C however, significantly enhanced their susceptibility to agglutination by con A (Table 1). No significant change (<10%) in ³H-con A binding to dibucaine-treated cells was detected. The susceptibility of 3T3 cells to agglutination by low doses of con A (350 μ g ml⁻¹) has also been enhanced by treatment for 1 h at 37°C with the local anaesthetics tetracaine (1 $\times$ 10⁻⁴ M) and procaine (1 $\times$ 10⁻² M).

Some effects of local anaesthetics on membranes are believed

Table 1 Effect of dibucaine on cell agglutination by con A

Treatment	Concentration of con A (μ g ml ⁻¹) for maximum cell agglutination*			
	3T3	SV3T3	BHK	PY-BHK
Untreated control	1,400	50	850	80
Dibucaine (1 $\times$ 10 ⁻⁴ M)†	350	50	100	50
Dibucaine (1 $\times$ 10 ⁻⁴ M) plus 5 mM CaCl ₂ ‡	1,250	50	850	80
Dibucaine (1 $\times$ 10 ⁻⁴ M) plus 10 mM CaCl ₂ ‡	1,400	50	850	80

* 90% of cell population agglutinated as determined by microscopic counts after incubation of cells with con A for 20 min at room temperature using methods described previously⁴.

† Cells were incubated in suspension in medium containing 1 $\times$ 10⁻⁴ M dibucaine (K and K Laboratories, Plainview, New York) for 1 h at 37°C before testing for their con A agglutinability.

‡ Cells were incubated in suspension in medium containing 1 $\times$ 10⁻⁴ M dibucaine supplemented with 5 or 10 mM CaCl₂ for 1 hour at 37°C before testing for their con A agglutinability.

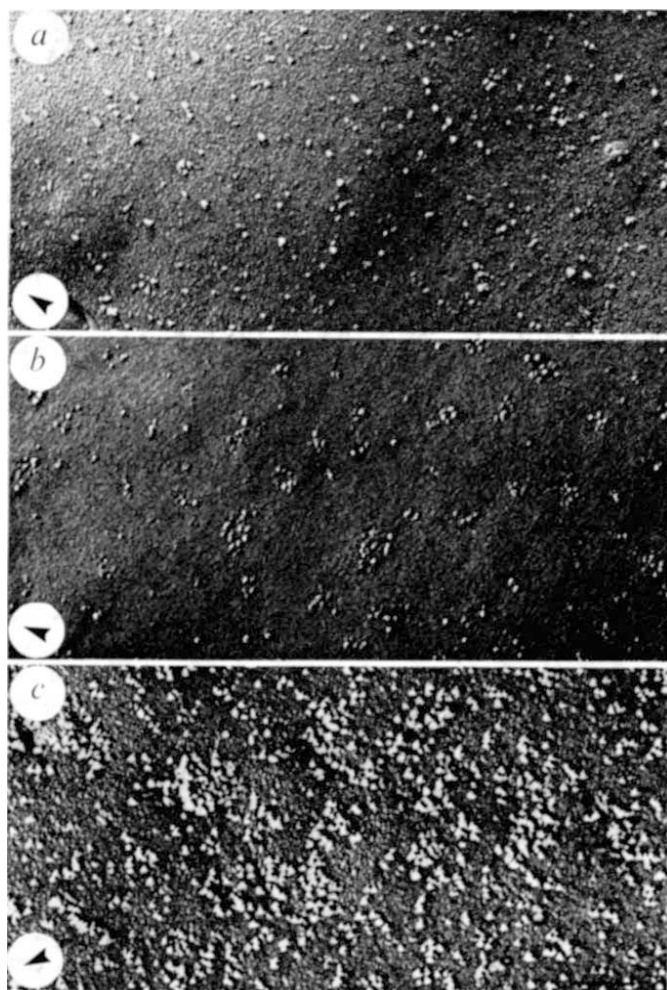


Fig. 1 Freeze-fracture electromicrographs (A face) of mouse 3T3 cells (100,000). The bar represents 0.1 μm . The arrows in the lower left corner of each micrograph indicate the direction of shadowing. *a*, Area of untreated control 3T3 cell showing predominant monogranular distribution of intramembranous particles. *b*, Area of 3T3 cell after incubation for 1 h at 37°C in medium supplemented with 1×10^{-4} M dibucaine showing increased clustering of intramembranous particles. *c*, Area of 3T3 cell after incubation for 1 h at 37°C in medium supplemented with dibucaine followed by incubation with 500 μg con A ml^{-1} for 15 min at 37°C, showing even more marked clustering of intramembranous particles than in (*b*).

to result from their ability to displace Ca^{2+} associated with the anionic groups of acidic phospholipids¹², and certain effects of these drugs can be inhibited competitively by increasing the Ca^{2+} concentration¹³. Similarly, incubation of 3T3 or BHK cells in medium supplemented with dibucaine plus additional Ca^{2+} (5 mM or 10 mM CaCl_2) eliminated the effect of dibucaine on cell agglutination by con A (Table 1).

Freeze-fracture observations of the surface of untreated 3T3 cells revealed a random and mostly single distribution of intramembranous particles (Fig. 1*a* and Table 2). After incubation with dibucaine, clusters of more than five particles were more common (Fig. 1*b* and Table 2). When similar dibucaine-treated cells were exposed to con A, more than 75% of the particles were clustered (Fig. 1*c* and Table 2). Thus, dibucaine causes a change in the structural organisation of the plasma membrane in 3T3 cells which is reflected in freeze-fractured images as redistribution of intramembranous particles, and this change is exaggerated in the presence of con A.

Electron spin resonance measurements on natural membranes¹⁵ and model membranes composed of phospholipid bilayers¹⁶ have shown that local anaesthetics increase the fluidity of phospholipids. Similarly, our data (in preparation)

from fluorescence polarisation measurements on dibucaine-treated cells—using the plasma membrane probe, diphenyl hexatriene⁷—suggests that dibucaine causes a small but significant increase in the fluidity of membrane lipids. It is not known whether this is a small change in the overall fluidity of the bulk lipid matrix or a much larger fluidity change in specific regions of the membrane. Differential scanning calorimetry and fluorescence polarisation studies on model bilayer membranes indicate that dibucaine causes a marked fluidising effect on several types of phospholipids (in preparation). At dibucaine concentrations (1×10^{-4} M) that enhance cell agglutination, however, this effect is confined to acidic phospholipids. This effect is more marked in acidic phospholipid membranes stabilised previously by Ca^{2+} , indicating that increased fluidity can also result in part from the displacement of membrane-bound Ca^{2+} by dibucaine. The same concentration of dibucaine caused no significant fluidity change in neutral phospholipids such as lecithin which constitute most of the plasma membrane lipids in mammalian cells. Thus, in intact cells low concentrations of dibucaine might induce a large fluidity change in specific membrane domains containing acidic phospholipids, without significant change in the fluidity of the bulk membrane lipid. Although such domains have not been demonstrated, experiments with mixed lipid bilayers indicate that Ca^{2+} can induce segregation of acidic phospholipids into separate domains from neutral phospholipids^{17,18}.

Table 2 Effect of dibucaine and con A on the density and distribution of intramembranous particles on freeze-fracture faces of 3T3 cell membranes

Treatment	Density of intramembranous particles (No. per μm^2)	Single	Distribution of intramembranous particles† (%)	
			Clusters < 5	Clusters > 5
Untreated control	495 $\pm$ 39	44.8	38.0	17.2
1×10^{-4} dibucaine‡	476 $\pm$ 23	21.9	41.6	36.5
1×10^{-4} dibucaine plus 500 μg con A §	515 $\pm$ 35	23.5	28.7	47.8

* Mean value from counts of intramembranous particles on two or more 0.1- μm^2 surface areas of A fracture face of individual 3T3 cells at a magnification of 100,000 and counts on at least fifteen individual cells. These values are in good agreement with those reported by Scott *et al.*¹⁴.

† Percentage of total number of intramembranous particles.

‡ Cells were incubated with dibucaine for 1 h at 37°C.

§ Cells were incubated with dibucaine for 1 h at 37°C followed by addition of 500 μg ml^{-1} con A and further incubation for 15 min at 37°C.

In addition to the fluidising effect, displacement of Ca^{2+} might induce changes in the organisation of peripheral proteins on the inner face of the plasma membrane which have been suggested to act as regulatory restraints in determining the movement of receptors on the outer face of the plasma membrane¹⁹⁻²². For example, spectrin, which is involved in the trans-membrane control of lectin receptor site distribution in erythrocytes²⁰, is released from membranes by Ca^{2+} -chelating agents²³. Moreover, spectrin interacts with acidic phospholipids²⁴ at pH 7.4 only in the presence of Ca^{2+} . By competing for such Ca^{2+} -binding sites dibucaine could affect the interaction of peripheral spectrin-like proteins with membrane lipids.

The two mechanisms proposed here whereby local anaesthetics displace Ca^{2+} from acidic phospholipids and alter the function of certain peripheral proteins on the inner face of the plasma membrane could operate in parallel or in tandem. A framework of proteins on the inner membrane face exerting trans-membrane control of the topography of receptor moieties on the outer membrane face could be linked, as in the case of spectrin, to acidic membrane lipids by Ca^{2+} . This type of organisation could provide a mechanism for changes in the mechanical

properties of the plasma membrane regulated by Ca^{2+} , which would not require constant or extensive changes in the overall fluidity of the bulk lipid matrix of the membrane.

When this work was being prepared for publication, Ryan *et al.*²⁵ reported that local anaesthetics inhibited antigen-induced capping of immunoglobulin molecules on the surface of B lymphocytes. They found, however, that the formation of patches of Ig molecules, which is the process analogous to clustering of con A receptors on non-lymphoid cells, was not inhibited by local anaesthetics, but no data were given as to whether patching on lymphocytes was actually enhanced.

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able to surmise that this asymmetry characterises the host cell plasma membrane as well. Thus, the consistency between our present findings and previous findings with red cell membranes⁹⁻¹⁵ suggest that an asymmetric phospholipid distribution of this type may be a general feature of plasma membrane structure.

The WSN strain of influenza A₀ virus was grown in Maden bovine kidney cells, and purified as before^{18,20}, except that a constant specific activity of ³²P-inorganic phosphate (2-5 $\mu\text{Ci ml}^{-1}$) was maintained in the medium throughout the cycle of cell growth and virus infection and multiplication. This assured that all phospholipid synthesised during this period had identical specific activity. Spikeless particles, from which the glycoproteins had been removed by treatment with bromelain, were prepared as before^{16,20}.

PLase C of two specificities was used. PLase C from *Clostridium welchii* (Sigma) was dissolved (0.5 mg ml^{-1}) in 0.05 M Tris, 0.02 M CaCl_2 , pH 7.4, and heated at 95°C for 15 min before use²¹. PLase C from *Bacillus cereus* (Sigma) was dissolved (40 U ml^{-1}) in 0.05 M Tris, 0.01 M CaCl_2 , 0.01 M MgCl_2 , pH 7.4, and heated at 56°C for 30 min before use. One volume of enzyme was added to 4 volumes of viral particles in 0.05 M Tris, pH 7.4, and the mixture was maintained at 37°C for 60 min. These conditions were chosen after trichloroacetic acid (TCA) precipitation had shown that 45-55% of the total phospholipid was hydrolysed and additional incubation caused no further phospholipid hydrolysis. The virions were then re-sedimented in a 5-40% potassium tartrate gradient at 35,000 r.p.m. for 90 min in a Spinco SW 50 rotor. The enzyme-treated particles, whether intact or spikeless, and their respective untreated controls sedimented similarly and seemed unchanged when negatively stained specimens were viewed in the electron microscope. The repurified virions were then extracted by the method of Folch *et al.*²². The extract was separated on silica gel thin-layer plates (Eastman Chromagrams) using the solvent chloroform-methanol-acetic acid-water (25:15:4:2)^{23,24}. After development, fractions from the plates were scraped into vials and radioactivity was counted. Approximately 90% of the total ³²P of the virions was recovered in the organic phase after extraction, and recovery of counts from the plates exceeded 95% of those applied.

Table 1 Hydrolysis of phospholipids from intact and spikeless influenza virions by PLase C

Phospholipid	% Hydrolysed		
	<i>Cl. welchii</i> enzyme	<i>B. cereus</i> enzyme	
Intact			
Sph	51	71	0*
PC	80	84	83
PS + PI	0*	0*	37
PE	43	56	42
Total hydrolysis	43	55	46

*Assumed as basis for calculation.

Asymmetry of influenza virus membrane bilayer demonstrated with phospholipase C

IN the red cell, the membrane carbohydrates¹, proteins²⁻⁹ and lipids⁹⁻¹⁵ are all unequally distributed between the two sides of the plasma membrane. The membrane proteins and carbohydrates of influenza virions are also asymmetrically distributed^{16,17} (review in ref. 18). We have now found that the phospholipids of influenza virions are asymmetrically distributed as well. Using phospholipase C (PLase C) to digest the phospholipids exposed on the outer surface we have found a predominance of phosphatidyl choline (PC) and sphingomyelin (Sph) on the outer surface and a predominance of phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) on the inner surface. Since influenza virions acquire their lipid bilayer by budding out from the membrane of their host cells, it is reason-

In Fig. 1a and b intact particles treated with the two types of PLase C are compared with their respective untreated controls. Figure 1c shows spikeless virus particles treated with the *Cl. welchii* enzyme^{16,20}. The results are tabulated in Table 1, based on normalisation to 100% recovery (that is, no hydrolysis) of PS after treatment with *Cl. welchii* enzyme^{14,25} and 100% recovery of Sph after treatment with *B. cereus* enzyme¹⁵. Values of total hydrolysis obtained in this way (Table 1) are in good agreement with the values obtained by TCA precipitation of unhydrolysed phospholipids.

The patterns of hydrolysis by the two enzymes are quite similar if differences in the inherent specificities are considered. Both enzymes hydrolysed 80-83% of the PC of the intact virion and 42-43% of the PE. *Cl. welchii* enzyme, hydrolysed 51% of the Sph from the intact virion, but 71% was removed from the spikeless particle. On the other hand, only 37% of the PS was hydrolysed by the *B. cereus* enzyme. This pattern of hydrolysis does not reflect the specificity reported previously for

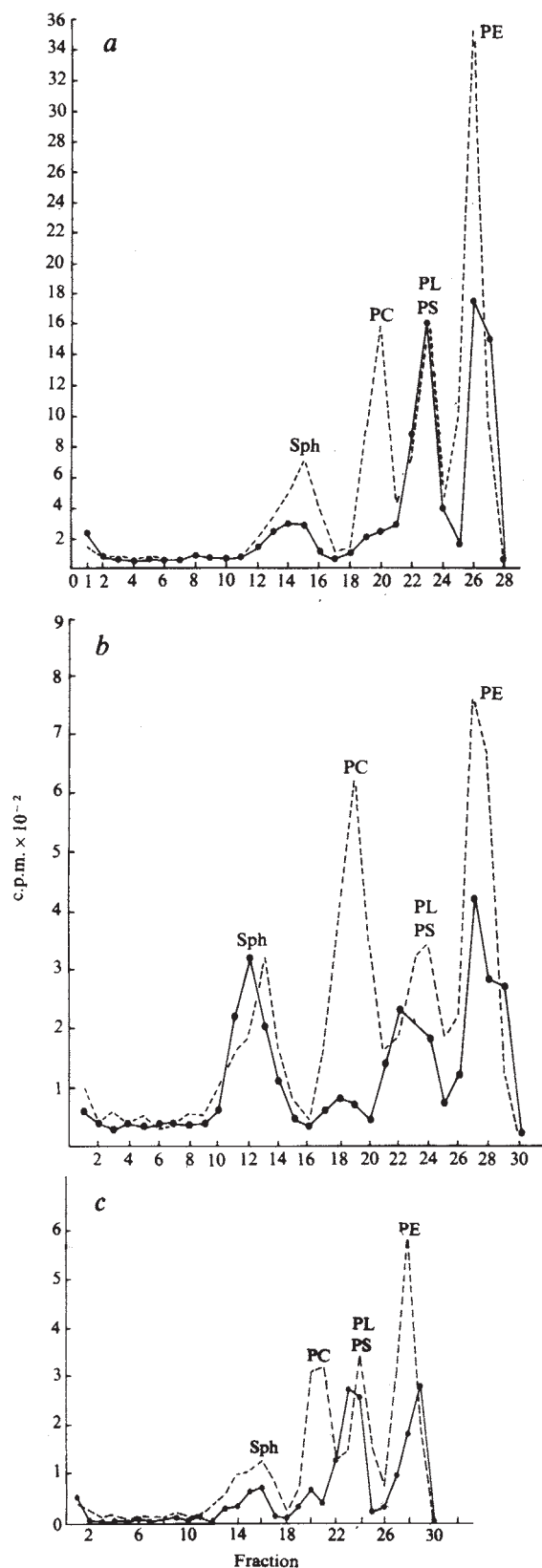


Fig. 1 Thin-layer chromatograms of phospholipid extracts of influenza virions after treatment with PLase C. Extracts from enzyme treated and control preparations were chromatographed separately, but are plotted together to facilitate comparison. — — —, Untreated control, — — —, PLase C-treated. *a*, Intact virions treated with *Cl. welchii* enzyme; *b*, intact virions treated with *B. cereus* enzyme; *c*, spikeless particles treated with *Cl. welchii* enzyme.

these two enzymes, both of which have been reported to be fully active against PE, while the *B. cereus* enzyme is also active against PS^{14,15,25}. Indeed, the PE and PS in the particles are almost completely hydrolysed by the *B. cereus* enzyme under appropriate conditions, for example after prolonged storage resulting in particle deterioration, or if divalent cations are omitted from the reaction medium. The overall similarity between results obtained with intact and spikeless particles shows that the results do not reflect the protection of particular phospholipids by the glycoprotein spikes of the intact virion.

The interpretation of these results in terms of asymmetry of the viral bilayer depends critically on the assumption that the virion is impermeable to PLase C. Influenza virions are known to be impermeable to proteases and ribonuclease, strongly suggesting that they are impermeable to other enzymes as well. Further, our results were obtained only with virions which were repurified after treatment with PLase C, thus ensuring that any disrupted particles were removed before lipid analysis. Finally, the observation that additional time of incubation, or incubation with additional enzyme, produced no additional hydrolysis suggested that a stable situation was reached in the particle in which the unhydrolysed phospholipids were unavailable to the enzyme. The penetration of the enzyme to the inner surface of the viral bilayer thus seems very unlikely.

Although our results do not permit detailed quantitation of the lipid distribution of each side of the membrane, they indicate an asymmetric distribution. PC and Sph are preferentially located on the outer surface of the viral bilayer, where they are readily accessible to externally added PLase C, while PS and PE are less accessible to the enzyme, suggesting preferential localisation on the inner surface of the bilayer. We believe that more Sph than the 50–70% hydrolysed is located on the outer surface, since Sph is known to impart resistance to digestion by *Cl. welchii* enzyme²¹, and an increase in the total amount of Sph in the viral particle results in decreased hydrolysis of this phospholipid (J. L. and K.-H. T. unpublished). On the other hand, the finding that no more than 80–85% of the PC is susceptible to hydrolysis suggests the location of the remaining 15–20% on the inner surface, since this phospholipid is readily hydrolysed by both enzymes. The results also suggest that part of the PS and PE is located on the outer surface.

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Extraneural competition between different scrapie agents leading to loss of infectivity

MANY strains of scrapie agent have been isolated which differ in their biological properties, such as incubation period and type of brain lesion¹. We have shown previously that competition occurred between different scrapie agents intracerebrally injected into mice², indicated by an increase in the incubation period of the lethal scrapie agent when a different scrapie agent had also been injected to impede its pathogenesis. We did not establish whether the increased incubation resulted from replication of the lethal agent being hindered throughout or at particular stages of pathogenesis, or whether the increased incubation period resulted from some loss of effective titre.

The pathogenesis of different scrapie agents injected into mice can only be described as relatively 'quick' or 'slow' if the recipient mouse genotype is specified in terms of the gene *sin*c (alleles s7 and p7). On this basis, inbred mice can be injected with a 'slow' agent which can block, partially or completely, the pathogenesis of a 'quick' agent injected later by the same route. We have previously found that the effectiveness of blocking depends on the route of injection, the interval between injections and the particular strains and doses of agent used. We show that agent competition can be so effective in some circumstances that the 'quicker' agent, injected later, seems to take no active part in the disease and may have been entirely degraded or excreted.

RIII mice (3–4 weeks old) were injected intraperitoneally with either brain homogenate containing 22A agent, or with a similar dose of normal VM brain as controls. All mice received a second intraperitoneal injection of brain homogenate containing 22C agent, given either 100, 200 or 300 days after 22A (Table 1). The expected incubation periods for 22A and 22C given singly in these doses would be 550 days and 230 days, respectively. To calculate incubation periods when two agents are injected, we must be able to determine which agent eventually kills the mouse or, even, whether both share in this process. 22A and 22C are quite different in the intensity and distribution of brain lesions which they produce (ref. 3 and H.F., unpublished), and consequently the 'lesion profile' for each group in the competition experiment can be used to decide which origin to use for calculating incubation periods.

Table 1 Scrapie in RIIIrosines^{s7s7} mice injected intraperitoneally either with two different scrapie agents or with only one agent; the interval between injection of the two agents was varied

First injection:	Normal brain	22A
Second injection:	22C	22C
Injection interval (days)	Brain lesion profile	Brain lesion profile
	type	type
	Incubation period (days ± s.e.)	Incubation period (days ± s.e.)
100	22C 237(± 6 (7))	22A 554 ± 13 (6)
200	22C 232 ± 2 (7)	22A 557 ± 5 (9)
300	22C 231 ± 0 (5)	22A 561 ± 7 (7)

Number of mice in parenthesis. Doses: 22A agent–0.02 ml of 10⁻¹ saline homogenate from 22A-infected VM brain, containing 10⁵ VM intracerebral LD₅₀ units 0.02 ml⁻¹. 22C agent–0.02 ml supernatant (2,000g for 15 min) of 10⁻² saline homogenate from 22C-infected C57BL brain, containing 10^{3.7} C57BL intracerebral LD₅₀ units 0.02 ml⁻¹.

Table 1 shows that the 22A blocking injection was so effective that the second injection (22C) failed to infect the mice. The lesion profiles for the blocked and control groups are significantly different (method of least squares; $P < 0.001$) and the profile in the blocked animals confirms that 22A was responsible for killing the mice. If 22C does take an active part in the disease in the blocked groups, although subsidiary to 22A, we would expect variation between the results for the three sub-

groups with different intervals between injections: there was no evidence of such differences (incubation period $P > 0.6$; lesion profile $P > 0.35$). We do not yet have titration estimates in RIII mice of the number of intraperitoneal LD₅₀ units of 22A and 22C but the doses were at least 10^{-1.5} and 10⁻², respectively.

We regarded this as further support for the scrapie replication site hypothesis⁴, which proposes that replication of scrapie agents depends on a multimeric host site, to which different types of subunit are contributed by the two alleles of *sin*c, that there is a limit on the total available number of replication sites and that this total is relatively small. Agent competition is therefore envisaged as resulting from the agent injected first, having had the opportunity to occupy some or all the available sites, and thus blocking the access of agent injected later, even though the latter may be a much 'quicker' agent than the one already there. The total efficiency of blocking achieved in the above experiment also indicates that the rate of site turnover is low, and that production of new sites must be infrequent, unless the blocking agent has priority in access to them. The low turnover implies that the agent occupying the sites, and presumably also replicated there, must not, at any stage of the replication process vacate the sites, even transiently, to allow access of agent injected later.

Competition between scrapie agents able to replicate in mice, raises the question of whether site blocking could be achieved using related agents which do not seem to replicate in mice, such as the agents of kuru, Creutzfeldt-Jakob disease or transmissible mink encephalopathy. It may even be possible to block sites with simpler, non-replicating, molecules. To be of practical use in the control of such diseases, however, it must be assumed that the sites which these agents use are not essential to the normal health of the animal.

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Relaxing and inotropic effects of cyclic AMP on skinned cardiac cells

THE positive inotropic effect of catecholamines in cardiac muscle is assumed to result from an increase in the intracellular level of cyclic AMP (adenosine 3',5'-monophosphate)^{1,2}. Cyclic AMP may enhance the contraction by increasing the trans-sarcolemmal flux of Ca²⁺ during the plateau of the action potential^{3,4}, but the flux seems insufficient to activate directly the myofilaments and it is generally assumed that additional Ca²⁺ may be released from intracellular stores (ref. 5), possibly by a Ca²⁺-triggered release from the sarcoplasmic reticulum (SR)^{6–8}.

Whether the intracellular level of cyclic AMP can modulate this release of Ca²⁺ is not known. Furthermore, catecholamines increase the rate of relaxation in the intact myocardial tissue⁹. How cyclic AMP could mediate this relaxing effect has not been established, although studies in isolated cardiac microsomes suggest that cyclic AMP may enhance Ca²⁺ binding by the

SR¹⁰⁻¹³. Experiments on intact cardiac tissue cannot answer these questions, as both the relaxing and inotropic effects of catecholamines and cyclic AMP might be entirely the result of the modulation of Ca²⁺ fluxes across the sarcolemma³.

We removed the sarcolemma by microdissection from fragments of single cardiac cells (less than 15 μ m wide and 60 μ m long) obtained by the homogenisation of rat ventricular tissues⁸ (Fig. 1). This allowed the cyclic AMP added to the solution to be in direct contact with the SR and the myofilaments of these 'skinned' (sarcolemma-free) cells. The resulting inotropic and relaxing effects were observed on tension recordings obtained with a highly sensitive photodiode force transducer¹⁴ (Fig. 1).

The free Ca²⁺ concentration ([Ca²⁺]) in the bathing solution was buffered with ethyleneglycol-bis (β aminoethyl ether) N,N'-tetraacetic acid (EGTA). The same free [Ca²⁺] was obtained with either a low or a high total [EGTA] (Fig. 1).

In the presence of a slight buffering with 5×10^{-5} M total EGTA cyclic contractions were induced by the increase of free [Ca²⁺] from 2.24×10^{-8} M to 3.16×10^{-7} M (Fig. 1a). The amplitude of the cyclic contractions was much smaller when the same increase of free [Ca²⁺] was associated with an increase of total [EGTA] from 5×10^{-5} M to 1.5×10^{-4} M (Fig. 1b). In the presence of 2×10^{-4} M or 4×10^{-3} M total EGTA, no cyclic contractions were observed and a tonic tension was induced by the same increase of free [Ca²⁺] (Fig. 1c, d and e).

This experiment suggests that the cyclic contractions were caused by cycles of release and re-sequestration of Ca²⁺ by an intracellular pool of Ca²⁺ which was in competition for Ca²⁺ with the EGTA buffer present in the solution. When the total [EGTA] is high (4×10^{-3} M) any Ca²⁺ released or sequestered by this Ca²⁺ pool would not appreciably modify the free [Ca²⁺] present in the myofilament space. Thus the observed tonic tension represented the direct effect of the free [Ca²⁺] set in the solution on the myofilaments (Fig. 1d). With a total [EGTA]

just above that which inhibited the cyclic contractions (2×10^{-4} M), however, the Ca²⁺ stores were still capable of competing with the EGTA. This competition was inferred from the slower rate of tension rise and lower plateau of tension as compared with that obtained with 4×10^{-3} M total EGTA (Fig. 1c, e and d).

When 10^{-6} M cyclic AMP was added to the solution with 2×10^{-4} M total EGTA, a partial relaxation of the tonic tension was obtained and was followed by the development of cyclic contractions (Fig. 1f). Conversely, removal of the cyclic AMP from the solution resulted in an increase of the tonic tension and in the disappearance of the cyclic contractions (Fig. 1f). Similar results were obtained in all of the 22 experiments done according to this procedure. These results indicate that cyclic AMP increased the capacity for Ca²⁺ sequestration of the internal pool of Ca²⁺, so that it could compete with larger total [EGTA].

In the presence of a free [Ca²⁺] of 2.24×10^{-8} M with 5×10^{-5} M total EGTA, a skinned cell was quiescent (Fig. 2a). A slight increase of free [Ca²⁺] to 3.98×10^{-8} M was sufficient to induce a phasic contraction. Then the total [EGTA] was increased to 4×10^{-3} M so that the direct effect on the myofilaments of the free [Ca²⁺] set in the buffer could be studied in the same cell. A free [Ca²⁺] of 3.98×10^{-8} M (the same free [Ca²⁺] as that inducing the phasic contraction with 5×10^{-5} M of total EGTA) did not evoke any tension when the total [EGTA] was 4×10^{-3} M. Thus this free [Ca²⁺] was below the threshold for direct activation of the myofilaments. A free [Ca²⁺] of 3.16×10^{-7} M with 4×10^{-3} M total EGTA induced a tonic tension smaller than the phasic contraction obtained with a free [Ca²⁺] nine times smaller. This suggests that the phasic contraction was the result of a release of Ca²⁺ from the internal stores permitting the myoplasmic free [Ca²⁺] to reach a much higher value than that set in the buffer. This release of Ca²⁺ was induced by a slight variation of free [Ca²⁺]

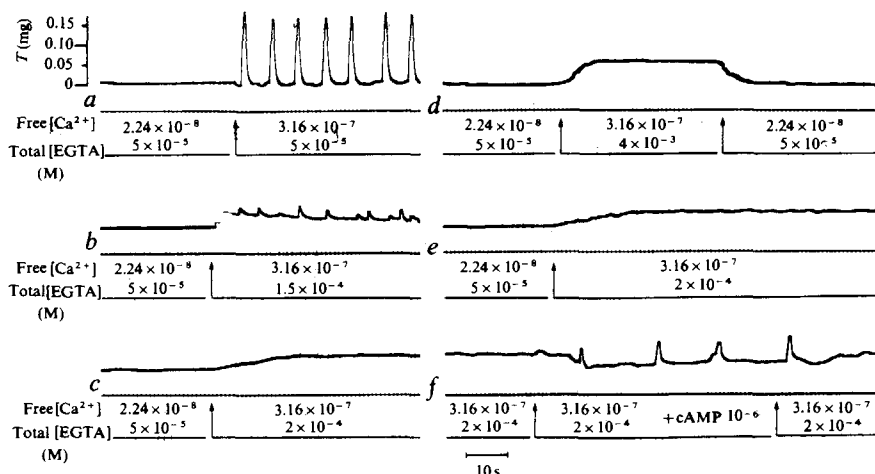


Fig. 1 Tension recording in a single skinned cardiac cell of 10 μ m width and 45 μ m length from rat ventricle. All tracings were obtained from the same cell. Tracings d, e and f are continuous. Arrows indicate solution changes. Adult rats (200 g) were decapitated. The heart was rapidly removed and the ventricular tissue was homogenised with a Virtis blender. An aliquot of the homogenate was placed in a perfusion chamber on the temperature controlled stage of an inverted Reichert Biovert Microscope. One end of a broken cell was immobilised with a glass microelectrode, while another microelectrode was used to pull the sarcolemma and superficial myofibrils from the cell. The two ends of the skinned cell were impaled with glass micro-tools (heat occluded microelectrodes) in a direction perpendicular to the axis of the myofibrils. The myofibrils developed strong adherence to the glass. One of the micro-tools was immobilised while the other was connected to the lever of a photodiode force transducer¹⁴. Perfusion was accomplished by several inlets (one for each solution) and one suction outlet. All media contained glucose 7 mM and Tris-maleate 18 mM, pH was 7.0 and the

temperature was maintained at 22°C. The concentrations of Na₂EGTA, CaCl₂, Na₂ATP and MgCl₂ were varied to obtain the desired free [Ca²⁺], while the free [Mg²⁺] was held constant at 3.16×10^{-4} M and [MgATP] at 3.16×10^{-3} M. The ionic strength was kept constant at 0.16 M by appropriate addition of KCl. The following apparent association constants were used at pH 7.0: Ca EGTA 4.9×10^6 M⁻¹; Mg EGTA 40 M⁻¹; Ca ATP 5×10^3 M⁻¹; Mg ATP 11.4×10^3 M⁻¹. If the binding of Ca²⁺ by ATP was ignored, a simplified expression of the free [Ca²⁺] would be:

$$\log_{10} \text{free } [\text{Ca}^{2+}] \approx 6.69 + \log_{10} \frac{\text{total } [\text{EGTA}]}{\text{total } [\text{calcium}]}$$

Thus the same free [Ca²⁺] can be obtained with various levels of total [EGTA] by appropriate variations of total [Calcium]. The cyclic AMP was obtained from Sigma Chemical Co., St. Louis, Missouri.

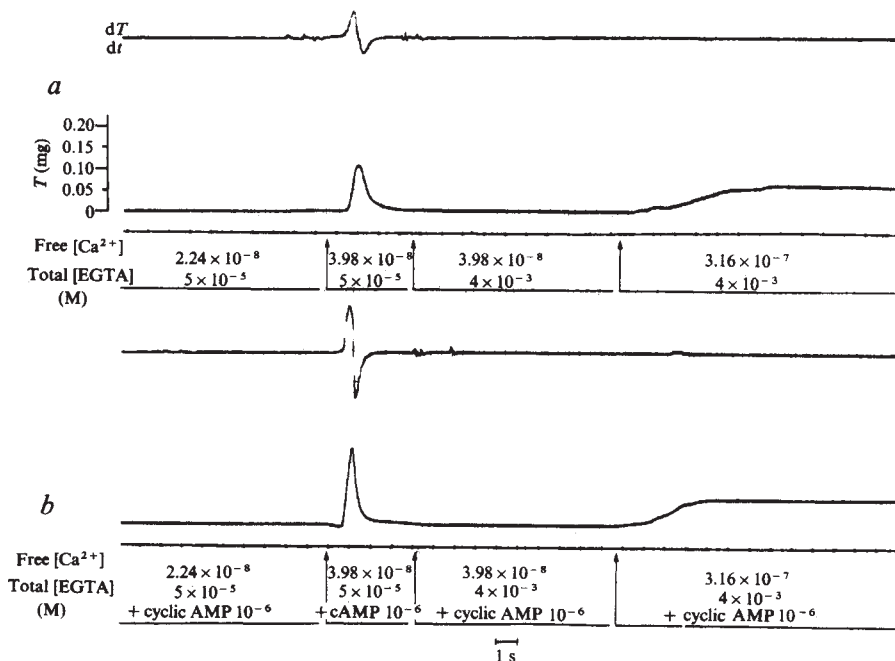


Fig. 2 Tension recording in a single skinned cell of 11 μm width and 52 μm length in the absence or in the presence of cyclic AMP. Arrows indicate perfusion changes. In addition to the experiments of the type represented in this figure, a curve of tension as a function of the free $[\text{Ca}^{2+}]$ was obtained, in the presence of 4×10^{-3} M total EGTA. The addition of 10^{-6} M of cyclic AMP did not change significantly any of the points on this curve.

in the buffer, consistent with a regenerative process⁶⁻⁸.

The same series of perfusions were repeated on the same cell with the addition of 10^{-6} M cyclic AMP to the perfusion media. The resulting phasic contraction was of larger amplitude (increase of $78\% \pm 19\%$ (SD) for 18 experiments) with shorter duration (decrease of $17\% \pm 9\%$) and faster rate of tension development and relaxation than in the absence of cyclic AMP (Fig. 2b). In contrast, no modification of the tonic tension obtained in the presence of a high total [EGTA] was observed (Fig. 2b). Addition of 10^{-2} M azide (inhibitor of the respiration linked uptake of Ca^{2+} by the mitochondria) and of 10^{-6} M ruthenium red (inhibitor of the passive binding of Ca^{2+} by the mitochondria) modified neither the phasic contraction nor the effects of cyclic AMP. In contrast, the destruction of the SR by the non-ionic detergent Brij 58 (ref. 15) eliminated the phasic contraction and the effects of cyclic AMP. Therefore, the effects of cyclic AMP on the phasic contraction and relaxation of the skinned cardiac cells seem to be related to modifications in the release and binding of Ca^{2+} by the SR and not by the mitochondria.

In conclusion, cyclic AMP has been shown to modulate contraction and relaxation of single cardiac cells in the absence of the surface membrane. The relaxing effect of cyclic AMP in skinned cardiac cells is probably because of an enhancement of the capacity (Fig. 1) and the rate (Fig. 2) of Ca^{2+} binding within the SR. Additionally, the release of a larger amount of Ca^{2+} from more fully loaded Ca^{2+} stores within the SR in the presence of a higher level of cyclic AMP may explain at least in part the positive inotropic effect of catecholamines. In contrast, no direct effect of cyclic AMP on the sensitivity to Ca^{2+} of the myofilaments was shown in the present study.

A.F. is an Established Investigator of the American Heart Association.

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Electrical activity and colonial behaviour in Anthozoan hard corals

ALTHOUGH colonial behaviour in the Anthozoa¹⁻⁴ has often been described, hypotheses relating the control of colonial polyp retraction to proposed nerve net activity have been based only on behavioural observations. This preliminary report of an electrophysiological investigation considers the conduction system controlling colonial polyp retraction in the Madreporarian coral *Porites porites* var. *clavaria*.

Single electrical stimuli of short duration at threshold level did not evoke any visible withdrawal response from any polyps. Two or more such stimuli applied at a single point within a second or two, however, always caused a colonial polyp retraction (Fig. 1). The retraction response was restricted to those polyps near to the point of stimulation and reached a maximum area of spread which was not exceeded even when large numbers of stimuli were applied⁴. A colonial polyp retraction in response to a single shock could only be evoked when stimuli of longer duration were used.

A single stimulus at threshold level evoked a single impulse which was conducted to large areas of the surrounding colony. There was no evidence for multiple firing in response to stimuli of this kind. Figure 2 shows a typical electrical response given by *P. porites*.

In the sea anemones^{5,6} and the Octocorallia⁹, available evidence suggests that the nerve net is responsible for the control of polyp retraction and that nerve/muscle potentials are the most easily recorded electrical events. This suggests that the electrical activity reported here for the colonial Hexacorallia also involves a nerve net/muscle response, although it is possible that the system may be neuroid. Electrical pulses could be stimulated by either mechanical or electrical stimuli.

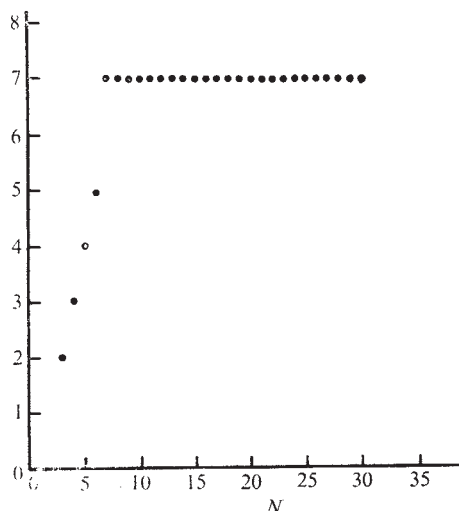


Fig. 1 Spread of colonial polyp retraction in *P. porites*, following repetitive electrical stimulation at a rate of 1 s^{-1} . r , radial distance of spread measured in polyp diameters; N , number of stimuli. Extracellular, polythene suction electrodes (see ref. 5) were used to record electrical activity. Similar electrodes were used to administer electrical stimuli. Each electrode was usually placed on the column or on the oral disk of a polyp.

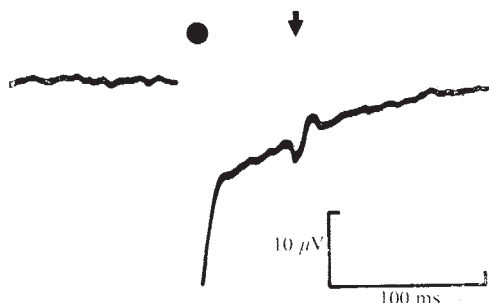
The conduction velocity in the non-fatigued conduction system was $15\text{--}20\text{ cm s}^{-1}$ at 25°C and the amplitude of the recorded pulses was small ($5\text{ }\mu\text{V}$ or less) with a threshold of $4\text{--}5\text{ V}$ for stimuli of 1 ms .

Colonial pulses are normally through-conducted, an observation at variance with predictions for many Anthozoan corals^{4,7}. These predictions were limited by the nature of the observations which could be made at that time and, based on behavioural data, assumed that nerve-net pulses did not spread significantly outside the area visibly shown to respond. The paradox that electrical activity is normally through-conducted although the spread of the behaviour is restricted, requires explanation.

Control of fast muscle contraction in the Anthozoa depends on the phenomenon of neuromuscular facilitation⁸. To bring about colonial polyp retraction, therefore, a number of pulses must be conducted across the colony. Furthermore, the frequency of the pulses will determine whether or not retraction occurs at any particular point, since this will determine the degree of facilitation of the neuromuscular junctions.

In response to a burst of stimuli at constant frequency, a corresponding burst of pulses was elicited but not at a constant frequency. It decreased in response to (1) increasing distance from the point of stimulation; (2) increasing number of pulses (Fig. 3). A number of explanations are possible for the progressively increasing conduction delays of successive pulses. If a nerve net is involved, there may have been progressively increasing synaptic delays, or the conduction path may have become longer and less direct. The reduction in frequency was most pronounced where the pulses were close together

Fig. 2 Recording of electrical activity (arrow) in *P. porites* following a single electrical stimulus (black spot).



in time; as the frequency of pulses decreased, the rate of slowing down decreased. The changes in conduction delay could account for the restriction of the area of polyp retraction because as the frequency of pulses progressively decreases the neuromuscular facilitation resulting from the preceding pulses decays before succeeding pulses arrive.

Horridge⁴ described patterns of polyp retraction in a number of colonial Alcyonarian and Madreporarian corals. He provided behavioural evidence showing that in some species (for example, *Favia*, *Coelaria*, *Goniastrea* and *Alcyonium*) all the polyps of the colony would retract in response to stimuli applied at one point, whereas in others (for example, *Porites*), the retraction response was restricted in spread and the whole colony was never involved in a colonial retraction response stimulated from one point. From these results, he developed a theory to predict the underlying nerve net events in terms of the 'density of active units' in a large array, and the probability of crossing from one unit to another. It was suggested that the size of contraction was related to the number of 'active units' (neurons or groups of neurons) in the nerve net at that point. Thus, in a region in which no contraction would take place, there was no reason to expect any significant amount of nerve net activity.

The results reported here fail to substantiate such a model or one based on the classic ideas of interneural facilitation. The different responses may be explained, however, as a function of the amount of delay introduced between electrical events conducted across the colony. The time intervals be-

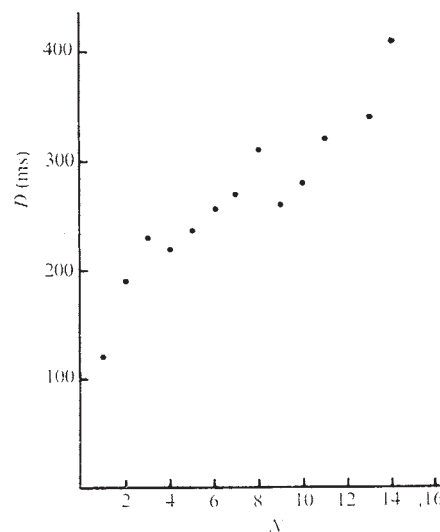


Fig. 3 Increase in conduction time with repetitive electrical stimulation at a rate of 1 s^{-1} . D , Conduction delay (ms); N , number of stimuli.

tween successive pulses determine the degree of facilitation of the neuromuscular junctions and this in turn controls the size of the muscular contraction evoked. Further data for colonial members of the sub-class Hexacorallia and for members of the sub-class Octocorallia will be reported elsewhere^{9,10}.

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Light autoradiographic localisation of cholinergic muscarinic receptors in rat brain by specific binding of a potent antagonist

THE presence of cholinergic muscarinic receptors on neurones in many regions of the mammalian brain is known from classical electrophysiological studies, which showed that neuronal firing rates are altered by the microiontophoretic application of cholinergic muscarinic agonists and antagonists¹. It is now possible to measure these receptors directly by biochemical procedures, some of which involve studying the responses of the cyclic guanosine monophosphate system in neuronal tissue to agonists and antagonists^{2–5}. Other procedures utilised radiolabelled muscarinic agonists and antagonists that bind specifically and directly to presumed receptor sites^{6–12}. One of these agonists^{13,14}, 3-quinuclidinyl-benzilate (QNB) binds in a radiolabelled form to apparent muscarinic receptors *in vitro*^{6,7}. Recent experiments indicate that it is also possible to demonstrate specific ³H-QNB binding to cholinergic muscarinic receptors in rat brain *in vivo* soon after intravenous administration¹⁵. Because of this latter factor, we have been able to examine the *in vivo* localisation of ³H-QNB in regions of rat brains by light microscopic autoradiography. We have found receptor sites associated with various groups of cells, some of which are known to be cholinceptive. In general, the greatest densities of ³H-QNB sites were in telencephalic regions.

Six male Sprague–Dawley rats (180–220 g) were administered 180 μ Ci of ³H-QNB (4 Ci mmol⁻¹) by tail vein injection in 0.3 ml of saline and killed by decapitation 1 h later. Three animals were pretreated with atropine (50 mg kg⁻¹, intramuscularly), 30 min before injection of ³H-QNB. It was necessary to utilise atropine-pretreated animals since all QNB is not displaceable by muscarinic drugs *in vitro* or *in vivo*. Only the displaceable portion is pharmacologically relevant and of interest here^{6,7,15}. There was no metabolism of ³H-QNB in the brain at the time of death¹⁵. The brains were removed rapidly from the skulls and 2–5 mm slices of the appropriate regions were mounted on copper block supports and quickly frozen by partial immersion in liquid nitrogen or, in later experiments, in a propane–propylene (10:1) mixture at liquid nitrogen temperatures. Frozen coronal sections (4 μ m thick) of these tissues were thaw-mounted^{16,17} on to emulsion-coated (Kodak NTB 3) microscope slides and stored desiccated for 30 d at 2° C. After exposure, the slides were brought to room temperature, developed, fixed, immersed in Carnoy's solution (a histological fixer) and stained with pyronine Y. After drying they were mounted with Permount and observed with a Zeiss Universal microscope. Control slides prepared for positive and negative chemography showed no evidence of significant fading of latent images or spurious generation of grains after 60-d exposures. The overall autoradiographic procedure utilised in this study has been described previously^{16–18}. We are also using other procedures^{17,19} which presumably would reduce further possible diffusion artefacts. Preliminary experiments, however, show results similar to those presented here. With regard to the problem of diffusion, a major factor in our favour is that the K_d for the QNB-receptor interaction is very low, 0.06 nM, and rate of dissociation of the QNB-receptor complex is correspondingly slow *in vitro*⁶ and *in vivo*¹⁵.

Of the areas examined, we found the highest densities of autoradiographic grains over the corpus striatum (nucleus caudatus-putamen), the cerebral cortex and the hippocampal

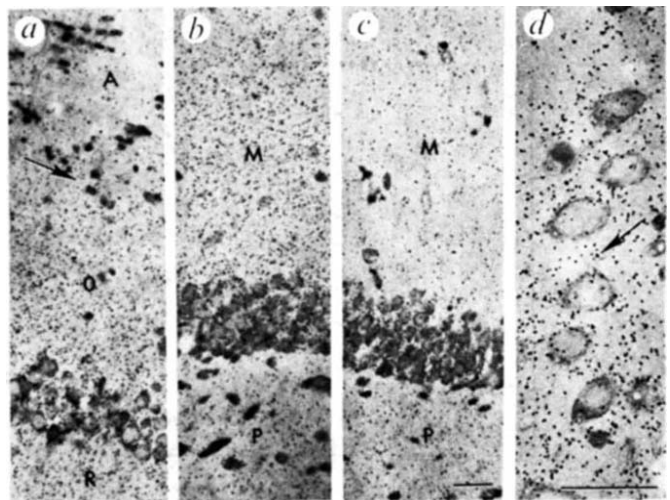


Fig. 1 Light autoradiographs of regions of the brain. The grains are shown most clearly in (d) (arrow), a higher power micrograph of the striatum. Lower power micrographs are shown in (a), (b) and (c) to make the pattern of distribution more observable. Bars represent 25 mm. *a*, Section of hippocampus proper showing pyramidal cells and adjacent areas in CA1. Note the high density of grains over regions on either side of the pyramidal cells, the stratum oriens (O) and the stratum radiatum (R), which contain the basal and apical dendrites of the pyramidal cells. The density decreases sharply at the boundary of (see arrow pointing along the boundary) and over the alveus (A), a fibre tract. *b*, Section of the dentate gyrus showing the granule cell layer and the adjacent areas, stratum oriens (O) and stratum polymorphe (P). The stratum oriens contains the dendritic processes of the granule cells, while the stratum polymorphe (this picture shows an area away from the pyramidal cells in CA4), is a heterogeneous zone containing the axons of the granule cells. Note the much higher density of grains in the region containing the dendritic processes. *c*, Same region as in (b) but from an atropine-pretreated animal. Note the great reduction in density of grains in the stratum oriens, the dendrite-containing region while there is little or no change in the stratum polymorphe. *d*, Higher magnification of a section of the striatum, a region with a similar density of grains as that of the dendrite-containing regions of the hippocampus. The density was relatively uniform across the entire nucleus caudatus-putamen.

formation (Fig. 1, Table 1). These results agree with our earlier *in vitro* and *in vivo* studies with rat brain^{6,15}. Apart from this more general evaluation, we were able to examine the distribution of grains in relation to specific types of cells known to have cholinergic muscarinic receptors. For example, the evidence is quite strong that acetylcholine is a neurotransmitter in septal afferents to the pyramidal and granule cells in the hippocampal formation^{20,21}. The cholinergic receptors on these cells are predominantly muscarinic, and most hippocampal cells were sensitive to acetylcholine, suggesting a widespread distribution of receptive areas^{22–24}. While choline acetyltransferase and acetylcholinesterase are distributed very similarly in discrete layers in the hippocampus, acetylcholinesterase staining boutons do not seem to be layered, but are scattered diffusely throughout the tissue, suggesting that cholinergic terminals are predominantly axo-dendritic²¹. Thus one would expect a widespread distribution of autoradiographic grains over regions containing dendrites of the pyramidal cells and perhaps also the granule cells. Our observations were consistent with these expectations. While these techniques do not permit visualisation of the dendritic processes, we found a high density of autoradiographic grains over the stratum oriens and stratum radiatum, regions containing the basal and apical dendrites of the pyramidal cells. The grain density seemed to be the same and relatively uniform in both of these regions, and was abruptly reduced at the boundary of and over the alveus, a fibre tract (Fig. 1a). The grain density was also greatly reduced in these areas in atropine-pretreated animals, indicating that most of the ³H-QNB binding in these

areas was the specific, pharmacologically relevant binding. The stratum moleculare of the dentate gyrus, a region containing the dendritic shafts of the granule cells, also exhibited a high grain density. The density decreased abruptly at the ventral boundary of the dentate gyrus and was reduced in the stratum polymorphe, a heterogenous zone containing the axons of the granule cells (Fig. 1b). The grain density in most sections was distributed evenly across the stratum moleculare, but in more posterior sections it was much higher near the granule cell bodies and much lower in the outer regions of the stratum moleculare. The density of grains in these regions was markedly reduced in sections from rats pretreated with atropine indicating the presence of many receptors (Fig. 1c). For example, when examined quantitatively, the stratum moleculare had 11.4 ± 0.65 grains per $100 \mu\text{m}^2$ in normal tissues, and 2.85 ± 0.53 grains per $100 \mu\text{m}^2$ in atropine-pretreated tissue. The background density found on the slides, off but near the tissue, was 0.33 ± 0.05 grains per $100 \mu\text{m}^2$ (data are mean $\pm$ s.e.m. from six slides of three animals).

We also sampled several other regions of the rat brain (Table 1). In the corpus striatum, a region where most cells are sensitive to iontophoretically applied acetylcholine²⁵⁻²⁷, and where there is also a high level of muscarinic receptors^{7,12}, we observed a high density of autoradiographic grains. This density was relatively uniform and high across the entire nucleus, suggesting a lack of clustering of cholinceptive cells. When examined quantitatively, the density of grains was more than four times higher between cells than over cells (12.9 grains per $100 \mu\text{m}^2$ compared with 2.96 grains per $100 \mu\text{m}^2$), again suggesting a localisation to dendrite processes (Fig. 1d). The density of grains was much diminished over adjacent areas of white matter such as the corpus callosum and the bundles of internal capsule fibres. Even though the density of grains in the white matter was very low, it sometimes seemed to be reduced by atropine, in agreement with *in vitro* studies^{7,12}. It has been suggested that the binding sites in white matter areas are receptors localised on axons of cholinceptive neurones¹².

In the cerebral cortex, the number of cells electrophysiologically sensitive to acetylcholine depends on the location explored^{28,29}. In this study, we observed variations in the density of grains in various regions of the cortex. There was a high density over cingulate areas and around cells in the pyriform cortex but a lower density at intermediate depths from the

surface (Table 1). In general, the density was greatly reduced in sections from atropine-pretreated animals, just as in the other regions.

The high densities observed in these regions differ from the lower density found around cells in various nuclei in the thalamus, hypothalamus, midbrain, medulla and cerebellum (Table 1). Perhaps cholinergic receptors in these regions are pharmacologically different from the QNB-type sites.

Thus it seems that one can associate these QNB binding sites with groups of cells in various brain regions. These findings would be useful for exploring the full significance and function of these presumed receptors. We are extending these studies so as to document more completely the distribution of these sites, to quantitate the distribution, and to examine the pharmacology of these presumed receptors in detail by electrophysiological methods.

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Antagonism of tolazoline by histamine H₂-receptor blockers

TOLAZOLINE (2-benzyl-2-imidazoline), a peripheral vasodilator and adrenergic blocking agent used to treat peripheral vascular disease and (formerly) hypertension, has actions similar to those of histamine, including vasodilation, pressor responses in the rabbit and stimulation of intestinal smooth muscle, gastric secretion and the heart. The cardiac stimulation has usually been referred to as sympathomimetic. Tachycardia and gastrointestinal distress are prominent clinical side effects of tolazoline. It has been used in place of histamine as a diagnostic agent for gastric function^{1,2}. The effects of tolazoline on gastric acid secretion and heart rate have, however, not been explained adequately in terms of mechanism or receptor theory. We have now been able to investigate this aspect using two specific H₂-receptor antagonists³, burimamide and metiamide. Our results indicate that the actions of tolazoline on sinus rate and

Table 1 Relative density of autoradiographic grains in representative brain regions

Region	Relative grain density
Hippocampus	
Stratum oriens	++++
Stratum radiatum	++++
Stratum moleculare	++++
Alveus	+
Striatum	++++
Cerebral cortex	
Cingulate cortex	++++
Pyriform cortex	+++
Intermediate depths of cortex	++
Thalamus	
Lateral thalamic nuclei	++
Nucleus parafascicularis	++
Medial habenular nucleus	++
Hypothalamus	
Arcuate nucleus	++
Dorsomedial nucleus pars ventralis	++
Midbrain	
Raphe nucleus	+
Interpeduncular nucleus	+
Cerebellar cortex	0 to +

Relative densities were determined by examining the brain regions after exposure for 30 d. Densities refer to specific binding as they are corrected for blank values obtained by examining similar regions from atropine-pretreated animals. Relative grain densities for cerebral cortex and striatum were measured on coronal sections of rat brain showing Brodard's area 6 of the cerebral cortex.

gastric acid secretion can be classified as H_2 -histaminergic. Recognition that tolazoline interacts with specific histamine receptors helps to explain why the drug has so many diverse actions affecting almost every organ in the body.

To investigate the influence of H_2 -receptor antagonists on tolazoline-induced gastric acid secretion we used female beagles with chronic gastric fistulae. Dogs were fasted overnight and prepared for intravenous infusion. Tolazoline was infused throughout the experiment, and gastric juice was collected every 15 min; the volume was measured and an aliquot was titrated with base to determine acid concentration. Burimamide or metiamide was dissolved in saline containing an equivalent of HCl and administered intravenously immediately after the seventh collection period (105 min) when secretion had reached a plateau.

Metiamide and burimamide, in doses which produced no other observable effects, significantly decreased both the gastric acid concentration and the volume secreted in response to tolazoline ($20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$). As Fig. 1 shows, metiamide ($20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) promptly decreased acid secretion for about 2 h, with a peak effect of nearly complete abolition of output. Half this dose elicited a similar response lasting about 1 h with a peak of 70% reduction in acid concentration. Burimamide ($20 \mu\text{mol kg}^{-1}$) also promptly decreased secretion for about 1 h, with a peak of 80% reduction. Thus, low doses of two H_2 -receptor antagonists markedly inhibited the near-maximal effect of tolazoline on gastric acid secretion. Under similar conditions, pyrilamine, an H_1 -receptor antagonist⁴ had no

Fig. 1 Effect of H_2 -receptor antagonists on gastric acid secretion induced by infusion of tolazoline in dogs. Tolazoline was infused intravenously continuously beginning at time 0 at a rate of $20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ in saline (20 ml h^{-1}). Effect of metiamide, $10 \mu\text{mol kg}^{-1}$ (○) and $20 \mu\text{mol kg}^{-1}$ (●), on acid concentration (mean $\pm$ s.e.m.; $n = 4$ each dose). Effect of burimamide, $20 \mu\text{mol kg}^{-1}$, on acid concentration (mean $\pm$ s.e.m.; $n = 2$).

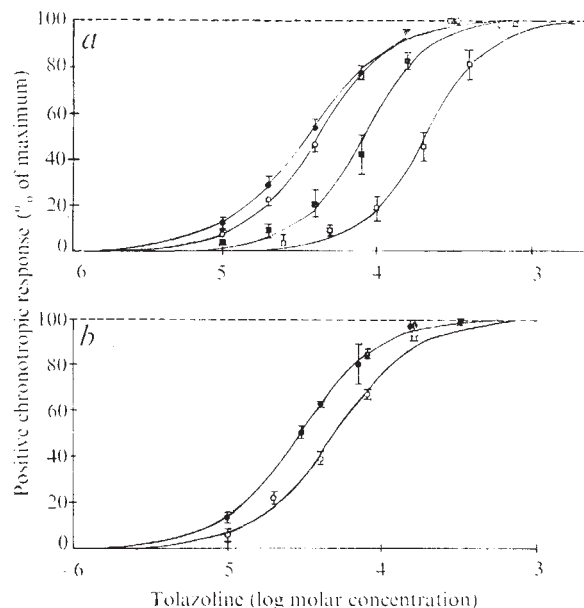
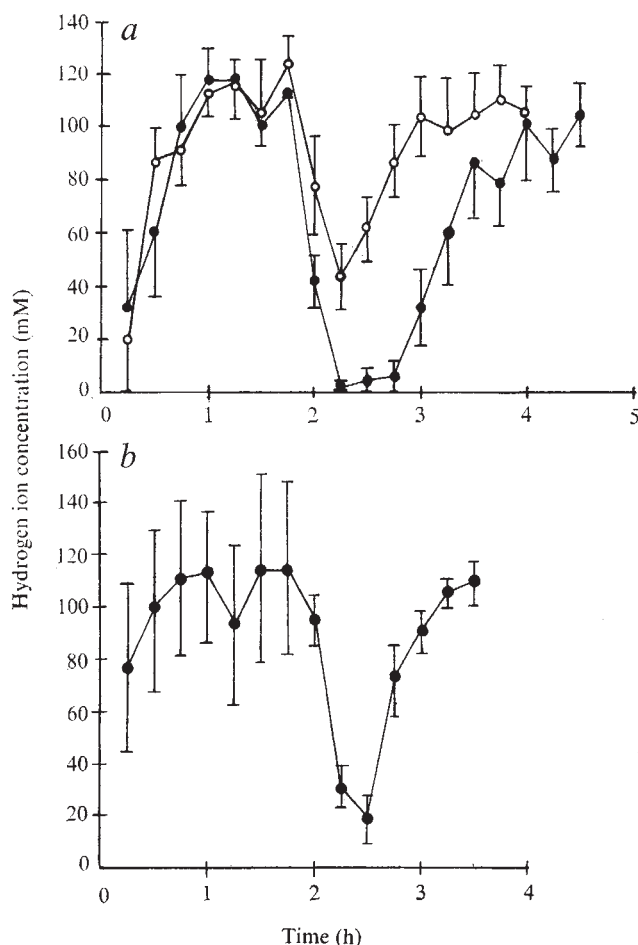


Fig. 2 Effect of H_2 -receptor antagonists on the positive chronotropic response of guinea pig atria to tolazoline. The nonlinear least squares estimation program NONLIN (ref. 8) was used to fit the log dose-response curves to the logistic function⁹

$$\frac{y}{Y} = \frac{c^n}{c^n + K^n}$$

where y = response, Y = maximum response, c = drug concentration giving response y , $K = \text{ED}_{50}$, and n = numerical function of the slope of the dose-response curve at the ED_{50} level. The mean response $\pm$ s.e.m. is plotted for each tolazoline dose. *a*, Response without antagonist (●; $n = 17$) and after equilibration with metiamide at $0.2 \times 10^{-6} \text{ M}$ (○; $n = 6$), $1.0 \times 10^{-6} \text{ M}$ (■; $n = 6$), and $4.0 \times 10^{-6} \text{ M}$ (□; $n = 6$). *b*, Response without antagonist (●; $n = 6$) and after equilibration with $2.0 \times 10^{-6} \text{ M}$ burimamide (○; $n = 6$).

effect. Our results suggest the involvement of H_2 -receptors in the secretagogue activity of tolazoline.

To investigate whether the cardiac effects of tolazoline might also be mediated by specific histamine receptors^{5,6}, and to provide more rigorous evidence on the mode of action of tolazoline, we studied the effects of tolazoline on heart rate in relation to cardiac H_2 -receptors identified by Black *et al.*³ and Parsons⁷. We measured the activity of tolazoline on contraction frequency of spontaneously beating atrial pairs, and its antagonism by metiamide and burimamide. Male guinea pigs (500–700 g) were stunned by a blow to the head and killed by exsanguination. The heart was removed immediately and placed in oxygenated (95% O_2 : 5% CO_2) Krebs–Henseleit buffer (pH 7.4). Both atria were dissected rapidly and suspended at 1 g tension in a thermostatically controlled (30°C) tissue bath (50 ml) of the same oxygenated buffer. Tissues were allowed to stabilise, with repeated washing, for 1 h. Individual contractions were recorded with a force-displacement transducer through a strain gauge coupler, and instantaneous rates were obtained with a cardi tachometer. Cumulative dose-response curves were constructed after sequential additions of tolazoline as a concentrated solution in saline so that the total added volume was less than 1% of bath volume. Heart rate was allowed to stabilise before addition of each subsequent dose. After a dose-response curve in the absence of antagonist had been obtained, tolazoline was washed out until the rate returned to baseline. The procedure was then repeated after equilibration (≥ 10 min) with the appropriate concentration of antagonist. The positive chronotropic response was defined as the increase over baseline rate just before addition of tolazoline. The baseline rate of 115 ± 2 beats per min ($n = 50$) was unaffected by burimamide or metiamide in the concentrations used. Maximal

rates in response to tolazoline (180 ± 3 beats min^{-1} ; $n = 26$) were invariably lower than those observed with histamine under the same conditions (211 ± 3 beats min^{-1} ; $n = 30$), and with tolazoline above $400 \mu\text{M}$, both rate and amplitude of contraction were depressed from their maxima. To normalise variations among tissues, responses are reported as percentage of the maximum increase, calculated for each preparation.

Metiamide, a competitive antagonist of histamine⁷, caused a parallel displacement of the tolazoline dose-response curves obtained with guinea pig atria (Fig. 2). Maximum responses were unchanged, and analysis of variance of the slope function, n , showed no significant difference in the slope of the curves. Ratios (DR) of doses needed for equal responses before and after equilibration with metiamide (Met) were calculated from the ED_{50} values and plotted according to the equation for competitive antagonism:

$$\log(\text{DR}-1) = \log(\text{Met}) - \log K_B$$

where K_B = apparent dissociation constant for the antagonist-receptor complex¹⁰. The slope of the linear regression of $\log(\text{DR}-1)$ on $\log(\text{Met})$ was 1.07, not significantly different from the theoretical value of 1. The K_B for metiamide, calculated according to Waud and Parker⁹, was $8.5 \times 10^{-7}\text{M}$ ($pA_2 = 6.1$) at 30°C , in good agreement with the value obtained by Parsons⁷ ($K_B = 9.2 \times 10^{-7}\text{M}$; $pA_2 = 6.0$ at 34°C) using histamine as agonist. According to Schild's criteria for classification of receptors^{11,12}, tolazoline and histamine appear to bind to the same receptors. As a point of interest, our ED_{50} values for the positive chronotropic response to tolazoline and to histamine were $35.2 \pm 1.2 \mu\text{M}$ ($n = 17$), and $1.2 \pm 0.2 \mu\text{M}$ ($n = 24$), respectively.

Burimamide also inhibited the positive chronotropic response to tolazoline. The curves shown in Fig. 2 were constructed from data obtained in two experiments using three atrial pairs in each. Burimamide at a concentration of $2.0 \times 10^{-6}\text{M}$ caused a parallel shift to the right of the dose-response curve by a factor of 1.8 and had no effect on the maximum response. The single dose ratio calculated from these data (assuming slope = 1, $pA_2 = 5.5$ at 30°C) falls within the 95% confidence limits reported by Black *et al.*³ for burimamide using histamine as agonist.

In addition to its effect on heart rate, tolazoline increased the amplitude of contraction which was antagonised by both burimamide and metiamide. Although we did not analyse the amplitude data quantitatively, it seems likely that the positive inotropic effect of tolazoline is mediated by H_2 -receptors, as shown for histamine¹³. The actions of tolazoline on cardiac rate and contractility were unaffected by the β -adrenergic receptor blocker, propranolol (10^{-6}M), or by two H_1 -receptor blockers, diphenhydramine and pyrilamine ($5 \times 10^{-6}\text{M}$ each).

In conclusion, these studies show that in addition to its well established activity as an α -adrenergic blocking agent, tolazoline is a histaminergic agonist. The use of this classification to describe tolazoline may provide the perspective needed to understand most of its pharmacological properties including the vasodilatory effects underlying its clinical usefulness. Indeed, we have shown that its pressor activity in the anaesthetised rabbit is abolished by pyrilamine unmasking a depressor response to tolazoline which is completely blocked by metiamide¹⁴. Furthermore, in the light of our evidence, previous investigations using tolazoline may require some reinterpretation because of a failure to distinguish between the adrenergic blocking activity and the histaminergic components of its actions. This may be particularly true of experiments in which tolazoline was used as a tool to dissect the actions of other drugs or to understand the functions of the adrenergic nervous system. In such cases the results can be ambiguous or misleading since in some tissues the activation of histamine receptors produces biological effects opposite to those of adrenaline while in other instances histaminergic and adrenergic effects are similar. Finally, our findings provide the medicinal chemist with fresh

clues for the design of histamine agonists and blocking agents.

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Identification of novel high affinity opiate receptor binding in rat brain

SPECIFIC opiate receptor binding sites¹⁻³, occur in the brains of numerous vertebrates. In our initial studies⁴⁻⁶ binding seemed to involve a homogeneous population of receptors, although the biphasic degradation of receptor binding by trypsin and chymotrypsin suggested more than one site⁷. We have used ³H-naloxone and ³H-dihydromorphine of very high specific activity, and now report evidence for a new high affinity opiate-binding site in addition to the already reported binding site of lower affinity. The high and low affinity binding site may reflect interactions of opiates with two conformations of the opiate receptor which determine agonist and antagonist actions of the drugs.

Male Sprague-Dawley rats (ARS Sprague-Dawley, Madison, Wisconsin, 180-220 g) were decapitated and their brains removed immediately. The cerebellum, which contains negligible binding¹, was excised and the remainder of the brain was placed immediately in the appropriate volume of iced standard Tris-HCl buffer (50 mM, pH 7.7 at 25°C) and homogenised with a Brinkmann Polytron for 60 s at setting 3. The homogenate was centrifuged for 20 min (49,000g, 0°C), the supernatant discarded, and pellets resuspended in the original volume of iced Tris-HCl buffer with a Brinkmann Polytron for 30 s at setting 3. Samples of 2 ml were then assayed with either ³H-naloxone (New England Nuclear Corp., 23.6 Ci mmol^{-1}) or ³H-dihydromorphine (New England Nuclear Corp., 53.6 Ci mmol^{-1}) at the stated concentrations and conditions, then filtered and counted as previously described⁵. Values are reported as stereospecific opiate binding⁸ and based on the means of triplicate determinations. All experiments were replicated at least three times. Values for the dissociation constants and number of binding sites were determined according to Klotz and Hunston⁷.

Scatchard plots of ³H-naloxone binding both in the presence and absence of sodium describe curves which can be resolved into two linear components with K_D values of 0.4 nM and 30 nM for high and low affinity binding respectively (Fig. 1a). Previously we reported that sodium increases the binding of ³H-naloxone and decreases the binding of ³H-dihydromorphine^{6,8}. When 100 mM sodium was included in the incubation medium, the number of apparent high affinity binding sites almost doubled, but the

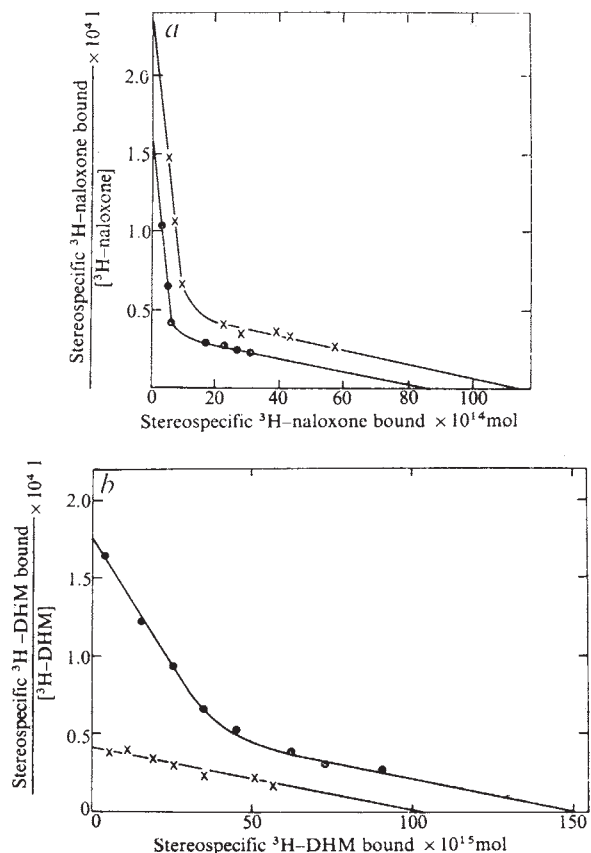


Fig. 1 Scatchard plots of ^3H -naloxone and ^3H -dihydromorphine binding in the presence and absence of NaCl. *a*, ^3H -naloxone Scatchard plot: 2-ml samples of brain homogenate (100 vol) were assayed in triplicate as described in the text, with various concentrations of ^3H -naloxone in the presence (x) or absence (●) of 100 mM NaCl and either (—) or (+)-3-hydroxy-N-allyl-morphinan at 0.1 μM . *b*, ^3H -dihydromorphine Scatchard plot: 2-ml samples of brain homogenate (100 vol) were assayed in triplicate, as described in the text, with various concentrations of ^3H -dihydromorphine in the presence (x) and absence (●) of 100 mM NaCl and either (—) or (+)-3-hydroxy-N-allyl-morphinan at 0.1 μM . All values for binding are stereospecific.

number of low affinity sites was unaffected and there was no significant effect on the dissociation constants. The increase in ^3H -naloxone binding elicited by sodium resembled our previous observations^{6,8}.

Biphasic Scatchard plots were also obtained for ^3H -dihydromorphine binding (Fig. 1 *b*). In the absence of sodium, high affinity binding for ^3H -dihydromorphine had a dissociation constant of about 0.3 nM, and low affinity binding exhibited a dissociation constant of about 3 nM. Sodium (100 nM) virtually abolished the high affinity binding of ^3H -dihydromorphine but had no apparent effect on the low affinity binding sites. Thus the effects of sodium on the binding of opiate agonists and antagonists are exerted predominantly on high affinity binding components.

The affinity of a ligand for its receptor is determined in part by its rate of dissociation from the receptor so that one would predict a slower dissociation rate for the novel high affinity binding site described here than was obtained for the lower affinity binding site reported previously⁴. Accordingly, after labelling the receptor with low concentrations (1 nM) of ^3H -naloxone we measured its dissociation from the receptor. Following the standard incubation the homogenate was centrifuged (49,000*g* for 20 min) and the pellet suspended in 10 times the original volume with 1 μM unlabelled naloxone incubated for various intervals at 0° C, filtered and counted. The half-life for dissociation of

naloxone was 30 min in contrast to a 5-min half-life reported at this temperature with higher concentrations of ^3H -naloxone labelling the 'low' affinity binding site⁴. The slower dissociation rate of lower concentrations of ^3H -naloxone is consistent with labelling of a novel binding site with high affinity.

In this study, using ^3H -naloxone and ^3H -dihydromorphine of high specific activities, we have detected a new opiate binding component with 100 times the affinity of ^3H -naloxone-binding sites described earlier^{1,4,5}. This high affinity binding, detected by using low concentrations of ^3H -opiates and ^3H -opiate antagonists, displays the same pharmacological specificity as binding using higher ^3H -drug concentrations^{1,4}.

What might be the functional significance of the high and low affinity opiate binding sites? We propose that they represent binding of opiates to two distinct conformations of the opiate receptor described recently⁸⁻¹⁰. The ability of sodium to increase binding of opiate antagonists and to decrease the binding of opiate agonists to the same extent implies that the opiate receptor exists in two forms. Antagonists have greater affinity for the antagonist-sodium form of the receptor while agonists bind preferentially to the agonist-no sodium receptor state. Pharmacological actions such as analgesia occur only when a drug binds to the agonist state of the receptor so that antagonists block the effects of opiate analgesics by binding to the antagonist conformation of the receptor, reducing the number of agonist forms of the receptor. The data presented here suggest that the high affinity binding of ^3H -naloxone involves the antagonist conformation of the receptor while low affinity ^3H -naloxone binding takes place to the agonist state of the receptor. Conversely, high affinity and low affinity ^3H -dihydromorphine binding respectively involve the agonist and antagonist states of the opiate receptor.

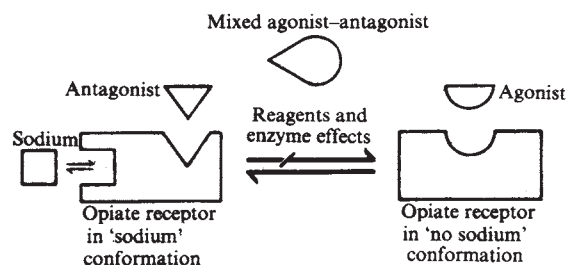


Fig. 2 A model of opiate receptor function.

While binding to the agonist form of the receptor is impaired by sodium, binding to the antagonist form should be unimpaired or increased. ^3H -dihydromorphine binding to high affinity sites is greatly reduced by sodium while its binding to low affinity sites is unaffected, which confirms our suggestion that low affinity binding of ^3H -dihydromorphine involves the antagonist state of the receptor. Our inability to demonstrate a marked sodium-induced enhancement in low affinity ^3H -dihydromorphine binding and a reduction in low affinity ^3H -naloxone binding may be the result of difficulty in measuring binding at these high ligand concentrations because of the high nonspecific binding and the difficulty in separating the high and low affinity binding sites by Scatchard analysis⁷.

We reported that several protein modifying reagents which attack sulphhydryl, tryptophan and other residues selectively decrease receptor binding of opiate agonists at low concentrations which do not affect antagonist binding^{9,10}. These reagents seem to interfere with the inter-conversion of agonist and antagonist conformation of the opiate receptor so that it freezes in the antagonist con-

formation. If, as postulated here, high and low affinity binding of ^3H -dihydromorphine respectively involve agonist and antagonist states of the receptor, protein modifying reagents should selectively impair high affinity binding of ^3H -dihydromorphine with no effect on low affinity binding. To test this idea, we examined the influence of iodoacetamide on ^3H -dihydromorphine binding at concentrations that reduce agonist but not antagonist receptor binding. In confirmation of this prediction, iodoacetamide markedly lowered high affinity binding of ^3H -dihydromorphine with no effect on its low affinity binding.

In summary, high and low affinity binding to the opiate receptor may reflect discrete interactions with the two conformational states of the receptor that account for the pharmacological properties of agonists and antagonists. The two binding sites may provide a valuable tool to investigate opiate receptor conformations.

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Prevention of the effects of fentanyl by immunological means

INVESTIGATIONS have demonstrated that the pharmacological effects of drugs such as digitalis¹ and paraoxon² can be altered by immunological means. To date, however, all reported attempts to antagonise immunologically the *in vivo* pharmacological effects of narcotic analgesics (such as morphine) have resulted only in mild attenuation or a partial delay in the onset of action³⁻⁵. To prevent immunologically the effect of a pharmacologically active molecule, sufficient amounts of specific antibodies must be present so that less than a threshold dose of the free drug remains after combination with the antibodies. Such a condition would be maximised with a drug which can be rendered highly immunogenic and which can exert its pharmacological effect at very low concentrations. We report that it is feasible to prevent the pharmacological effects of the potent narcotic analgesic fentanyl in experimental animals by both passive and active immunisation.

We have previously reported^{6,7} that high titres of antibodies of high specificity and affinity could be induced against the very potent synthetic narcotic analgesic, fentanyl. Fentanyl [N-(1-(2 phenethyl-4-piperidinyl) propionanilide)] has a spectrum of pharmacological effects similar to morphine, all of which can be antagonised by naloxone; however, fentanyl produces pronounced analgesia at 1/200 the dose of morphine.

Fentanyl, therefore, seemed to be a good candidate for investigating the neutralisation of the effects of an opiate-like drug by antibodies.

Antifentanyl antibodies were produced by immunisation of two rabbits with carboxyfentanyl [N-phenyl, N-(1-(β -phenethyl) piperidine succinamic acid)] conjugated to the protein carrier bovine gamma globulin (BGG). Conjugation was performed using 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide as previously described⁶, the conjugate containing 12 mol fentanyl per mol BGG. Immunisation was achieved by three bi-weekly subcutaneous injections of 2 mg conjugate in saline emulsified with an equal volume of Freund's complete adjuvant (FCA). The antigen binding capacity of the antisera produced was such that 0.25 μl serum could bind 0.005 μg and 0.0025 μg ^3H -fentanyl, respectively.

Table 1 Prevention of fentanyl analgesic effect by neutralisation of the drug *in vitro* and by passive and by active immunisation

Treatment	No. of mice	Hot plate end points (s $\pm$ s.e.m.)	
		Before treatment	90 s after treatment
Neutralisation*			
Fentanyl + saline	5	9.8 $\pm$ 0.99	> 60
Fentanyl + control serum	5	8.6 $\pm$ 0.99	> 60
Fentanyl + immune serum	5	6.8 $\pm$ 0.62	7.7 $\pm$ 1.62
Passive immunisation†			
Control serum followed by fentanyl	5	7.0 $\pm$ 0.57	> 60
Immune serum followed by fentanyl	5	7.4 $\pm$ 0.75	7.8 $\pm$ 1.12
Active immunisation‡			
Immunisation with fentanyl-BGG conjugate in FCA	15	8.0 $\pm$ 0.49	18.8 $\pm$ 5.6
Immunisation with FCA only	10	6.8 $\pm$ 0.54	> 60

*Fentanyl was administered at a dose of 100 μg kg^{-1} in a total volume of 0.2 ml per 30 g mouse. Fentanyl was incubated with saline, control serum or immune serum for 10 min at room temperature before intravenous administration. Saline, control serum and immune serum, when injected alone to control mice, did not alter the end point.

†Mice were given 0.5 ml control serum or immune serum intravenously and challenged with 100 μg kg^{-1} fentanyl (0.2 ml per 30 g mouse) 30 min later. Control serum and immune serum, when injected alone to control mice, did not alter the end point.

‡Specific immunisation was performed with fentanyl-BGG conjugate in Freund's complete adjuvant (FCA); controls were immunised with saline in FCA. Injections were given intraperitoneally, twice at 2 weeks interval. Challenge with 100 μg kg^{-1} fentanyl in a total volume of 0.2 ml per 30 g mouse was performed 14 d after the last injection.

Assessment of the ability of rabbit antifentanyl antibodies to bind *in vitro* with the drug and to neutralise its analgesic effect was carried out as follows. An amount of fentanyl citrate equal to three times the ED_{50} dose ($3 \times 33 \mu\text{g}$ kg^{-1}) was preincubated for 10 min with 0.3 ml specific rabbit antisera, 0.3 ml of rabbit antiserum to tobacco mosaic protein, serving as control serum, or with 0.3 ml saline and then injected intravenously to NAMRU mice⁸. The mice were subsequently tested for analgesia by the hot plate technique of Eddy and Leimbach⁹ using their criteria for end point determinations. Each mouse was tested on the hot plate before, and 90 s after intravenous injection. The results of these experiments show that intravenous administration of 3ED_{50} of fentanyl, either in saline or preincubated with control serum, produced analgesia in 100% of the tested mice. In contrast, when 3ED_{50} of fentanyl were preincubated with specific rabbit antifentanyl serum the analgesic effect of fentanyl was dramatically reduced (Table 1). We then ascertained whether prevention of drug effects could be accomplished by passive immunisation *in vivo*. Two groups of mice were given intravenous injections of either 0.5 ml of the rabbit antifentanyl serum or 0.5 ml control serum; 30 min

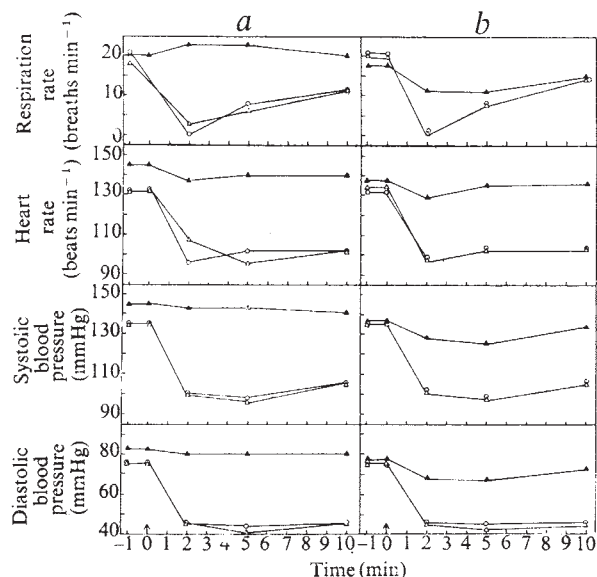


Fig. 1 Prevention of the effect of fentanyl in dogs. *a*, *In vitro* neutralisation: $5 \mu\text{g kg}^{-1}$ fentanyl were preincubated with 2 ml saline ($\circ$), 2 ml control globulins ($\triangle$) or 2 ml antifentanyl globulins ($\blacktriangle$) for 10 min at 37°C . The mixtures were given intravenously to two dogs at zero time ($\longrightarrow$). *b*, Passive immunisation: two dogs were injected intravenously with 2 ml saline ($\circ$), 2 ml control globulins ($\triangle$) or 2 ml immune globulins ($\blacktriangle$) 30 min before challenge with $5 \mu\text{g kg}^{-1}$ fentanyl at zero time ($\longrightarrow$).

later each mouse was challenged intravenously with 3ED_{50} of fentanyl. Table 1 shows that the analgesic effect of fentanyl was prevented only in mice receiving specific rabbit antifentanyl serum; the remaining animals exhibited analgesia. To determine whether active immunisation of mice would alter the analgesic effect of fentanyl, we immunised two groups of mice, one group receiving two intraperitoneal injections, 14 d apart, of $250 \mu\text{g}$ fentanyl-BGG conjugate in 0.1 ml saline emulsified with equal volume of FCA, the second receiving two identical injections of saline emulsified with FCA. Ten days after the second injection all the animals were bled from the tail vein, sera were collected and titres of antifentanyl antibodies determined by the Farr assay¹⁰ as follows: various dilutions of pooled sera were incubated for 60 min at room temperature with $0.01 \mu\text{g}$ of internally labelled ^3H -fentanyl. The complex was precipitated at 50% saturation of ammonium sulphate, washed three times with 50% saturated ammonium sulphate, the precipitate taken up in 10 ml scintillation fluid and the radioactivity determined on the Nuclear Chicago Mark I scintillation counter. The fentanyl-BGG-immune sera had titres to the extent that $1 \mu\text{l}$ could bind $0.0075 \mu\text{g}$ ^3H -fentanyl. Four days after serum collection the mice were challenged intravenously with 3ED_{50} of fentanyl. Table 1 shows that active immunisation prevented the analgesic response in mice challenged with 3ED_{50} of fentanyl.

To determine whether drug effects other than analgesia could also be prevented by antifentanyl antibodies, we monitored systolic blood pressure, diastolic blood pressure, respiration rate and heart rate in dogs. Preliminary experiments with two dogs weighing approximately 10 kg each showed that the intravenous administration of $2.5 \mu\text{g kg}^{-1}$ fentanyl produced marked effects on the selected parameters. Two other dogs were anaesthetised with halothane, and maintained at a minimal stable level of anaesthesia with 1.5% halothane. Neutralisation of the effects of fentanyl was attempted by preincubation of $5 \mu\text{g kg}^{-1}$ fentanyl with 2 ml control globulins, antifentanyl globulins or saline, followed by intravenous administration of the various mixtures into each of the dogs. Figure 1*a* shows that all the effects of the drug were prevented by antifentanyl antibodies, whereas the effects of the drug were fully expressed

(even to the extent that breathing had to be assisted) in the controls.

Passive immunisation was then attempted by administering 2 ml of antifentanyl globulins to two mongrel dogs (10 kg body weight) and then challenging 30 min later with $5 \mu\text{g kg}^{-1}$ of fentanyl. Figure 1*b* shows that only the administration of antifentanyl globulins had the capacity to prevent the effects of the challenging dose of fentanyl. Previous administration of either saline or control globulins had no effect on subsequent fentanyl administration.

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Liver tryptophan pyrrolase activity and metabolism of brain 5-HT in rat

TRYPTOPHAN is the amino acid precursor of 5-hydroxytryptamine (5-HT) both peripherally and in the central nervous system, but the metabolism of tryptophan by way of the 5-HT pathway accounts for only 1% of urinary tryptophan metabolites¹. The major route of tryptophan metabolism starts with its conversion to formylkynurenine by tryptophan pyrrolase², and it has been suggested^{3,4} that depressive illness may be caused by the induction of liver tryptophan pyrrolase by plasma corticosteroids⁵, leading to decreased synthesis and turnover of 5-HT in the brain. We confirm the increase in activity of liver tryptophan pyrrolase produced by hydrocortisone and the decrease in activity by allopurinol reported by Curzon and Green². Hydrocortisone also increased the concentration of 'free' tryptophan in the serum. Although the concentration of 5-HT in the brain was unchanged, tryptophan and 5-hydroxyindole acetic acid (5-HIAA) concentrations in the brain were also increased. 'Free' and total tryptophan concentrations in the serum were reduced by allopurinol injection, but concentrations of tryptophan in the brain were unchanged. These results are not compatible with the theory that the induction of liver pyrrolase by hydrocortisone induces the symptoms of depressions.

Male Sprague-Dawley rats (250–300 g) were housed at constant temperature under a 12 h light/12 h dark cycle. The effects of the injection of hydrocortisone (15 mg kg^{-1}) and allopurinol (20 mg kg^{-1}) on liver tryptophan pyrrolase activity were measured and related to changes in 'free' and total tryptophan concentrations in the serum, brain tryptophan, 5-HT and 5-HIAA concentrations (Table 1). Induction of the enzyme by hydrocortisone was maximal 3 h after injection¹. Total tryptophan concentrations in the serum remained

Table 1 Effect of hydrocortisone and allopurinol on pyrrolase activity and tryptophan metabolism

	Pyrrolase activity (μmol kynurenine $\text{h}^{-1} \text{g}^{-1}$)	Serum total tryptophan ($\mu\text{g ml}^{-1}$)	Serum free tryptophan ($\mu\text{g ml}^{-1}$)	Brain tryptophan ($\mu\text{g g}^{-1}$)	Brain 5-HT (ng g^{-1})	Brain 5-HIAA (ng g^{-1})
Control	5.84 ± 0.38 (6)	12.61 ± 1.06 (6)	2.05 ± 0.18 (6)	1.42 ± 0.06 (6)	444 ± 8 (8)	267 ± 9 (5)
Hydrocortisone (15 mg kg^{-1} intraperitoneally)	16.44 ± 0.55 (6) [†]	11.82 ± 0.54 (6)	4.81 ± 0.97 (6)*	1.64 ± 0.06 (6)*	407 ± 16 (10)	321 ± 12 (16)*
Control	6.73 ± 0.45 (4)	11.02 ± 0.5 (6)	3.69 ± 0.03 (6)	1.61 ± 0.07 (6)		
Allopurinol (20 mg kg^{-1})	2.67 ± 0.43 (4)*	6.33 ± 0.3 (6) [†]	2.01 ± 0.11 (6) [†]	1.43 ± 0.04 (6)*		

All animals were killed at approximately the same time of day to avoid the influence of the circadian variation of tryptophan, 5-HT and 5-HIAA in rat brain and serum. Total tryptophan in serum and brain was assayed by the method of Denckla and Dewey⁸, unbound tryptophan was assayed by the same method following equilibrium dialysis against Krebs-Ringer solution (pH 7.4). 5-HT was assayed by the method of Snyder *et al.*⁷, and 5-HIAA by the method of Giacalone and Valzelli⁸. Liver tryptophan pyrrolase activity was estimated by the method of Knox and Auerbach⁵.

* Significance at $P < 0.01$ level; [†] significance at $P < 0.001$ level.

Means $\pm$ s.e. are shown. Numbers of animals per group in parentheses.

unchanged but concentrations of 'free' tryptophan in the serum and brain tryptophan were significantly increased ($P < 0.001$). Brain 5-HT concentrations were unchanged but brain 5-HIAA concentrations were significantly increased ($P < 0.01$).

Liver tryptophan pyrrolase activity was significantly decreased ($P < 0.001$) by the injection of allopurinol (20 mg kg^{-1}) intraperitoneally. Concentrations of total tryptophan in the serum were simultaneously reduced by 60% and of 'free' tryptophan by 53%. The concentrations of tryptophan in the brain were also lowered.

When allopurinol and hydrocortisone were administered together the increased pyrrolase activity induced by hydrocortisone was reversed. No significant change in concentrations of tryptophan in the brain was measured but total and 'free' tryptophan in the serum were reduced by 60% and 30% respectively.

Corticosteroids have been shown to elevate free amino acids in the plasma as a result of protein breakdown in tissues other than the liver⁶, and to enhance synthesis of liver protein including the synthesis of liver tryptophan pyrrolase⁵. Concentrations of total and 'free' tryptophan in the plasma may therefore be expected to depend on the balance of these systems. Knott and Curzon⁹ have shown that the concentrations of tryptophan in the brain are correlated with concentrations of 'free' tryptophan in the plasma. Our results agree with this latter correlation since the concentrations both of 'free' tryptophan in the serum and of tryptophan in the brain were increased after hydrocortisone injection but total tryptophan concentrations remained unchanged. Since the concentrations of tryptophan in the brain are below the K_m for tryptophan hydroxylase¹⁰ it is not surprising that the increased concentrations of brain tryptophan led to an increased turnover of 5-HT in the brain.

The hypothesis developed by Curzon⁹ maintained that low concentrations of 5-HT in the brain were related to increased pyrrolase activity which in turn was caused by high corticosteroid concentrations. This hypothesis depends on the assumption that the induction of liver pyrrolase by corticosteroids results in a decreased availability of tryptophan for conversion to 5-HT in the brain. The effects of hydrocortisone demonstrated in these experiments make this theory untenable, although the possibility that corticosteroids may participate in altering the metabolism of 5-HT in the brain cannot be discounted, particularly in light of recent evidence which suggests the presence of a pyrrolase enzyme in the brain¹¹.

It is also possible that, as suggested by Curzon, long term activation of pyrrolase could cause a functional deficiency of pyridoxal in the brain, or that elevated levels of tryptophan

metabolites formed on the pyrrolase pathway could have secondary effects on 5-HT levels¹².

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Increased UDP kinase activity in rat and human hepatomas

PREVIOUS work¹⁻⁴, (carried out in this laboratory with the molecular correlation concept as a conceptual and experimental approach resulted in an insight into the biochemical strategy of the cancer cell) much of it conducted with a model system of hepatomas of very different growth rates⁵, has revealed a gradually emerging metabolic imbalance that is linked with the increase in tumour growth rate^{1-4,6-12}. Such biochemical alterations were manifested in progressive changes in the activities of opposing and competing key enzymes and opposing and competing metabolic pathways that correlated closely with tumour growth rate. These alterations in gene expression that seem to be linked with the increase in the expression of malignancy were manifested in increases in the activities of key glycolytic, pyrimidine and DNA synthesising enzymes^{4,7-12}. Concurrently, there were progressive decreases in the activities of the key enzymes of gluconeogenesis, pyrimidine catabolism and the urea cycle^{4,7,8,10,12}. In addition to this 'malignancy-linked' (growth-rate-linked) metabolic imbalance, we now show that the reprogramming of gene expression in cancer cells entails 'transformation-linked' alterations that are present in all hepatomas irrespective of growth rate or differentiation.

Our investigations on the UTP-synthesising enzyme, UDP kinase (nucleosidediphosphate kinase; ATP:UDP phosphotransferase, EC 2.7.4.6) were undertaken because in the hepatomas of different growth rates the capacity of certain key enzymes involved in the biosynthesis of UDP increased in parallel with cell proliferation rate¹³⁻¹⁵. Concurrently, utilisation of UDP was potentiated by an enhanced activity of ribonucleoside-diphosphate reductase (EC 1.17.4.1)⁹. Quantitatively smaller increases were also observed in differentiating and regenerating liver^{11,13} (for reviews see refs 11 and 14-16). Since the reductase and the UDP kinase compete for the UDP pool, their strategic metabolic positioning suggests that a critical imbalance in the reprogramming of gene expression may be present in the spectrum of hepatomas of different growth rates (Fig. 1). Our studies with various hepatomas of vastly different growth rates and differentiation showed that specific activities of UDP kinase were markedly increased in all neoplasms irrespective of tumour proliferation rate or degree of differentiation. This increased potential for UTP biosynthesis should provide a mechanism by which the cancer cell can adjust the utilisation of UDP for RNA and deoxyribonucleotide biosynthesis for subsequent formation of DNA.

Male Buffalo and ACI/N rats were maintained as described previously^{4,7}. Preparation of regenerating liver, studies on developing animals, killing of rats and excision of livers and tumours were as reported elsewhere^{4,7,12}. The growth rates of the various hepatoma lines ranged from 2 weeks to 11.5 months, measured by the time required for the tumours to reach a diameter of 1.5 cm.

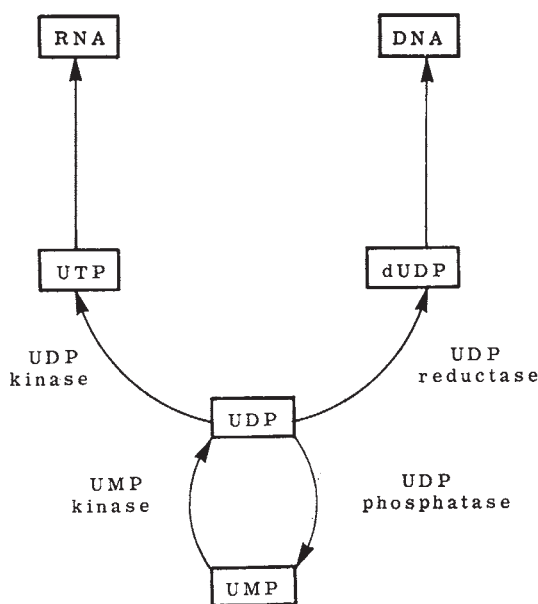


Fig. 1 UDP metabolism. This simplified schematic diagram indicates the strategic position of UDP in pyrimidine, RNA and DNA metabolism. The role of the four enzymes involved in UDP production and utilisation is noteworthy. For further details on the behaviour and interrelationships of the key enzymes and metabolic pathways in liver neoplasia, see review by Weber⁴.

Kinetic studies were carried out to ensure proportionality of UDP kinase activity with amount of enzyme added. A standard enzyme assay system was developed that was adapted to the kinetic conditions of the rat liver and hepatoma systems. UDP kinase specific activity was expressed as μmol of substrate metabolised $\text{h}^{-1} \text{mg}^{-1}$ protein. The details of the assay and a comparison of the specific activity and protein content are given in Fig. 2.

The kinetic characteristics of UDP kinase activity from crude enzyme preparations of rat liver and the rapidly growing hepatoma 3924A were similar. The pH optima were broad,

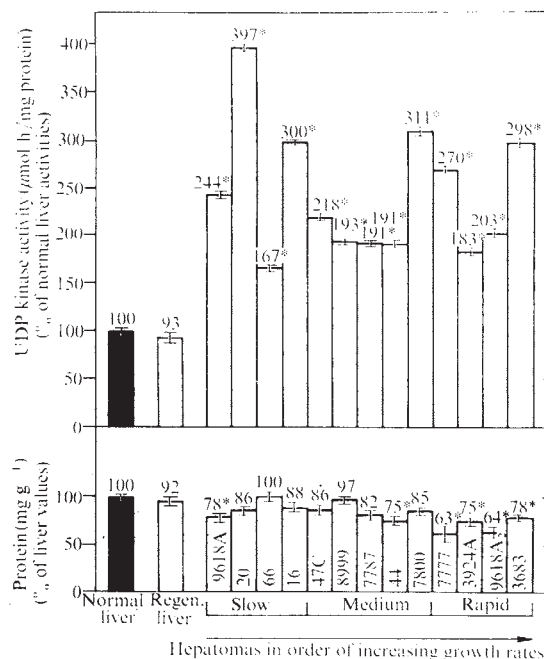


Fig. 2 Increased uridine-5'-diphosphate kinase (UDP kinase) activity in rat hepatomas of different growth rates. One per cent homogenates (w/v) were prepared at 4°C from liver and tumour tissues in 0.15 M KCl, pH 7.4, which were made 5 mM dithioerythritol (DTE) after protein determination. Supernatant fluids (100,000g) were used for the determination of UDP kinase activity as measured by the conversion of ^3H -UDP to ^3H -UTP. The incubation mixture contained in a total volume of 0.2 ml, 50 μmol of glycyl-glycine, pH 7.5; 7.5 μmol of MgCl_2 ; 1.5 μmol of β -mercaptoethanol; 7.5 μmol of ATP (diNa), pH 7.4; 2.5 μmol of UDP; 1.5 μCi of uridine-5- ^3H -5'-diphosphate ammonium salt (19.6 Ci mmol^{-1}); and 2 μl of a 1:4 dilution of 1% supernatant fluid. Enzymatic activity was determined by withdrawing three 30- μl samples at 2 min intervals and spotting directly on PEI-cellulose plates (Brinkmann), activated with 10% NaCl, at 65°C (hot air blower). The PEI-cellulose plates were washed for 10 min in 500 ml of anhydrous methanol and air dried. The product of the reaction (UTP) was separated from the substrate (UDP) chromatographically with 4.0 M HCOONa , pH 3.4, in glass rectangular tanks. No nucleoside-diphosphate (EC 3.6.1.6) activity was detected under these conditions. In this system the UTP spot was located (at the origin) with ultraviolet light, marked with a soft lead pencil, cut out with scissors and placed in counting vials with 10 ml of scintillation fluid (0.03% *p*-bis (2-5-phenyloxazolyl) benzene and 5% 2,5-diphenyloxazole in toluene from New England Nuclear Corp. ^3H -UTP was determined in a Packard Tri-carb (Model 3390) liquid scintillation spectrometer. Protein concentration was assayed by the Folin method of Lowry *et al.*¹⁹, using recrystallised bovine serum albumin as a standard. One unit of UDP kinase activity will produce 1 μmol of UTP in 1 h at 37°C and pH 7.4. Specific activity was expressed as units per mg protein. UDP kinase activities of the hepatomas are compared with corresponding control normal liver values. The UDP kinase activity of the normal liver was 444 ± 9 μmol per h per mg protein. The data are means $\pm$ s.e. of four or more animals in each group and the results are plotted as percentages of corresponding control liver values. The protein content was calculated as mg protein per gramme wet weight of tissue and it is also given in percentages. The measurement of growth rates of the individual hepatoma lines was discussed elsewhere⁴.

*Values statistically significantly different from the respective controls ($P < 0.05$).

ranging between 7.0 and 9.0. The apparent K_m values for UDP, ATP and MgCl_2 at pH 7.4 and 37°C were 0.5, 3.0 and 3.0 mM, respectively.

UDP kinase specific activities were markedly increased in all neoplasms irrespective of tumour growth rate or degree of differentiation. The specific activities in the slowly growing hepatomas 9618A, 20, 66 and 16 were 2.4, 3.9, 1.7 and 3.0 times greater, respectively, than those in the corresponding normal rat livers. In hepatomas of medium growth rates 47C, 8999, 7787, 44 and 7800 the activities were 2.2, 1.9, 1.9, 1.9 and

3.1 times higher than those in normal liver. In the rapidly growing neoplasms 7777, 3924A, 9618A2 and 3683, activities were 2.7, 1.8, 2.0 and 2.9 times greater, respectively, than those of normal liver.

In the 24-h regenerating liver, UDP kinase activity was similar to that in liver of sham-operated control rats. Enzyme activity in the average cell of 6-d-old rat liver was in the same range as that of the normal rat (data not shown). Since increased UDP kinase activity was present in the hepatomas, but not in rapidly growing differentiating or regenerating liver, the higher activity of this UTP-synthesising enzyme seems to be specific to neoplastic transformation. This tentative conclusion was supported by studies on two primary human hepatomas. Using the histologically normal samples from the human host liver as controls, we observed that UDP kinase specific activity in the two human hepatomas was increased 2.3 and 4.9 times.

Our studies demonstrate a marked imbalance in hepatoma UDP utilisation. Whereas UDP kinase activity was increased in all hepatomas irrespective of the rate of cell proliferation (Fig. 2), the ribonucleoside-diphosphate reductase activity from the very low levels of the liver (less than 2 pmol per h per mg protein) increased markedly in parallel with tumour growth rate⁹. Thus, the regulatory mechanisms for gene expression are reprogrammed differently for these two UDP-utilising enzymes. The reductase activity, as it is increased in parallel with the growth rate, is linked with the different degrees in the expression of malignant properties. In contrast, the increased UDP kinase activity, as it is present in all hepatomas, even in the most slowly growing well differentiated neoplasms, seems to be linked with the neoplastic transformation *per se*.

Our demonstration of high UDP kinase activity in hepatomas, irrespective of the degree of malignancy, differentiation or growth rate, suggests that the reprogramming of gene expression in malignant transformation is linked with an increase in the expression of this UTP-synthesising enzyme activity. In this laboratory recently there were discovered four such transformation-linked increases in enzyme activities, and they all relate to an increased potential in the routing of precursors to strategic biosynthetic processes. Thus, the increase in the activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and transaldolase (EC 2.2.1.2) provides an increased potential for channelling glycolytic intermediates into pentose phosphate biosynthesis¹⁷. The third alteration observed in all hepatomas was an increase in amidophosphoribosyltransferase (EC 2.4.2.14) activity which should also result in an increased potential for purine and nucleic acid biosynthesis¹⁸. In turn, the greater UDP kinase activity reported here should provide an increased potential for RNA and DNA biosynthesis.

These increases in the activities of key enzymes of ribose-5-phosphate biosynthesis, purine production and the increase in UDP kinase activity indicate a reprogramming of gene expression that should confer selective biological advantages to the neoplastic cells.

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Role of precursor 16S RNA in assembly of *E. coli* 30S ribosomes

RECONSTITUTION of bacterial ribosomes occurs *in vitro* in a system containing ribosomal components only¹, which suggests that ribosomes also form by self-assembly *in vivo*. This inference is reinforced by the fact that intermediary particles, containing a similar fraction of ribosomal proteins, are observed both *in vitro* and *in vivo*¹⁻³. The *in vitro* reconstitution process, however, requires a very high energy of activation, presumably to force the intermediates into a rare conformational state^{1,4}. This requirement is too high to be compatible with the rate of ribosome formation *in vivo*⁵. Furthermore, there is evidence that the rate-limiting step which leads to the accumulation of the precursor particles *in vivo* does not involve spontaneous conformational change of these particles⁶. Although all the information, therefore, required to construct a ribosome must be present in its components, it seems that cells use an additional mechanism to facilitate their assembly.

We report that reconstitution of 30S particles occurs *in vitro* with a much lower energy of activation if the precursor form of 16S RNA (p16S)⁷ is used in place of the mature RNA (m16S), and if the RNA is complemented with ribosomal proteins (r-proteins) extracted from nascent rather than from pre-existing ribosomes. We suggest that to facilitate ribosome assembly bacterial cells use a morphopoietic factor which recycles between precursor particles and completed ribosomes in concomitance with the maturation of rRNA.

The ionic conditions of Traub and Nomura⁵ were used both to dissociate and to reassociate ribonucleoprotein particles. A mixture of p16S and m16S RNA, differentially labelled with ³H- and ¹⁴C-uracil and obtained from particles sedimenting on a sucrose gradient at 25-30S, was incubated with increasing amounts of r-proteins extracted from total ribosomes. Incubation was carried out for various times both at 0°C and at 40°C to determine the temperature dependence of the reconstitution of 30S particles.

To obtain an efficient incorporation of rRNA into 30S particles a twofold excess of 30S proteins had to be used. In these conditions reconstitution of 30S particles occurred only at 40°C (Fig. 1a). At 0°C both p16S and m16S RNA formed particles sedimenting at 20-22S (Fig. 1b). Thus in the standard conditions of Traub and Nomura p16S RNA is no more efficient than m16S RNA in forming ribosomal subunits, confirming a recent observation⁸.

When the r-proteins/rRNA ratio in the incubation mixture was increased to a value greater than 3, some p16S RNA was incorporated into particles sedimenting at 30S even at 0°C. The amount of ³H-labelled 30S formed at 0°C did not increase with the time of incubation. Incorporation was the same when the mixture was analysed in sucrose gradients after an incubation of 2, 20 or 60 min, although it did increase with the amount of r-proteins added. At a protein/RNA ratio of 10 to 12, all p16S RNA was incorporated into 30S particles at 0°C and m16S RNA formed particles sedimenting at about 25-26S

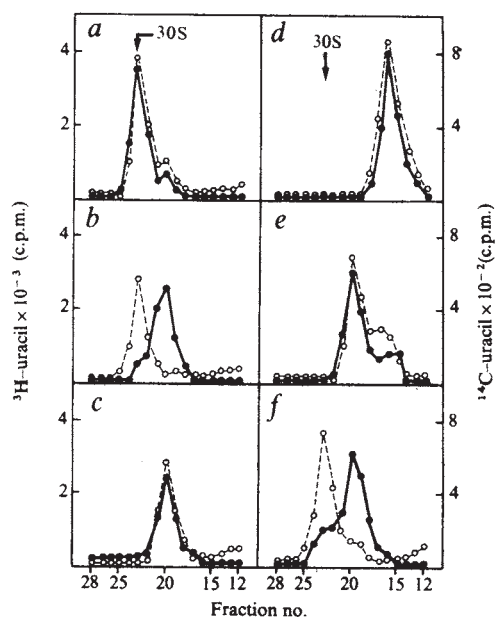


Fig. 1 Reconstitution of 30S particles from p16S and m16S RNA in the presence of different amounts of r-proteins. ^3H -labelled p16S and ^{14}C -labelled m16S RNA were obtained from *E. coli* D10 cells growing in MS9 medium supplemented with 0.2% glucose and 0.5% casamino acids¹⁰. The culture (20 ml) was labelled with 1 μC ^{14}C -uracil ($\bullet$) for two generations and with 150 μC ^3H -uracil ($\circ$) for the last 2 min of growth. Cells were extracted at an A_{420} of 1.0 by pouring on to crushed ice, collected and lysed as previously described¹⁰. The lysate (1 ml in 10^{-2} M MgCl_2) was centrifuged on a sucrose gradient in 10^{-4} M MgCl_2 for 7.5 h in an SB238 International rotor at 40,000 r.p.m. The 30S peak was localised by counting an aliquot of each gradient fraction. Fractions containing material sedimenting from 25 to 30S were pooled and mixed with an equal volume of 8 M urea, 6 M LiCl. After 24 h the RNA was pelleted by centrifugation and redissolved in 0.3 M KCl, 20 mM Tris-HCl (pH 7.6), 2 mM MgCl_2 , 6 mM β mercaptoethanol and dialysed against the same buffer. An aliquot of RNA was precipitated with ethanol, redissolved in 1% sodium dodecyl sulphate and analysed using polyacrylamide gel electrophoresis as described previously⁹. The majority (90%) of the ^3H -labelled RNA (p16S) moved as a single sharp peak 6–8% slower than the ^{14}C -labelled RNA (m16S). Ribosomal proteins were extracted by the same LiCl-urea treatment both from total ribosomes and from native subunits and 70S particles. In the first case a crude extract of alumina ground cells was layered on top of a 5 ml sucrose gradient in 10^{-2} M MgCl_2 , and centrifuged long enough to pellet all ribosomal particles including native subunits and their precursors. In the second case, native subunits and 70S ribosomes were displayed on a regular sucrose gradient in 10^{-2} M MgCl_2 , localised by the A_{260} absorbance and collected by pelleting from the corresponding fractions. The pellets were resuspended in 4 M urea, 3 M LiCl, and 24 h later RNA was removed by centrifugation and supernatants were dialysed against 1 M KCl, 20 mM Tris-HCl (pH 7.6), 20 mM MgCl_2 , 6 mM β mercaptoethanol. To prepare the reconstitution mixture a sample of the RNA preparation was mixed at 0°C with the volume of protein preparation required to obtain the desired r-protein/rRNA ratio. KCl concentration was then reduced to a final value of 0.3 M by adding the appropriate volume of a buffer containing 20 mM Tris-HCl (pH 7.6), 20 mM MgCl_2 and 6 mM β mercaptoethanol. The relative amounts of RNA and protein were calculated from the volume of culture from which they had been obtained. After incubation at the temperature and for the time indicated below, the reconstitution mixtures were centrifuged on sucrose gradients in 10^{-3} M KCl, 10^{-2} M Tris-HCl (pH 7.6) for 7 h at 40,000 r.p.m. to display 30S particles and reconstitution intermediates formed. *a*, Protein from total ribosomes added in a ratio of 2:1 with RNA, incubated at 40°C for 20 min; *b*, as *a* but incubated at 0°C for 60 min; *c*, protein from total ribosomes added in a ratio of 12:1; incubated at 0°C for 5 min; *d*, protein from 70S ribosomes in a ratio of 4:1, incubated at 0°C for 5 min; *e*, protein from 70S ribosomes in a ratio of 30:1, incubated at 0°C for 5 min; *f*, protein from native subunits in a ratio of 4:1, incubated at 0°C for 5 min. The direction of sedimentation was from right to left.

(Fig. 1c). ^{14}C -labelled 30S particles containing m16S RNA appeared at 0°C only at a protein/RNA ratio of about 30.

^3H -labelled 30S particles formed *in vitro* at 0°C starting from p16S RNA were capable of responding to T4 specific messenger RNA by entering polyribosomes when incubated in conditions for protein synthesis (data not shown; for details on the assay see refs 6 and 9). This response was observed even when the assay was run at a temperature as low as 18°C to avoid the exposure of reconstituted particles to 37°C . On the basis of their ability to respond to T4 messenger RNA, together with their sedimentation properties, therefore, ^3H -labelled reconstituted particles resembled native 30S subunits and differed from the ^{14}C -labelled intermediary complexes formed in the same reconstitution mixture starting from m16S RNA.

p16S RNA can thus form 30S particles in the cold more efficiently than m16S RNA. Several possibilities may be advanced to suggest why this does not happen in the presence of stoichiometric amounts of r-proteins but only in the presence of a large excess. Some ribosomal proteins could be partially inactivated during the extraction procedure. At 40°C , however, a protein/RNA ratio of 2–3 was sufficient to convert all rRNA into 30S particles, indicating that the putative inactivation was reversed by heating. Preheating r-proteins at 40°C before mixing with the RNA, however, did not relieve the high ratio requirement for the reconstitution of 30S at 0°C . Second, at 0°C the 20–22S complex formed could have a much lower affinity for the missing proteins than at 40°C . When the RNA concentration was also increased twenty-five to thirtyfold, that is, when stoichiometric amounts of RNA and proteins were incubated at a very high concentration, no 30S formation occurred.

Third, a factor present only on a minor fraction of ribosomes could lower the energy of activation of 30S assembly, allowing it to occur rapidly at 0°C . To test this hypothesis, we extracted proteins from 70S ribosomes and from native ribosomal subunits separated by sedimentation on a sucrose gradient. The preparation of ribosomal subunits included ribosomal precursor particles. As shown in Fig. 1d and e, proteins extracted from 70S ribosomes did not sustain the formation of 30S particles in the cold even at a protein/RNA ratio of 30. A complete incorporation of p16S RNA into 30S particles was instead obtained with proteins extracted from native subunits at a ratio of 4 (Fig. 1f).

The assembly of 30S particles *in vitro* may therefore be facilitated by a factor which can be extracted with the proteins of newly formed or precursor ribosomal particles, but is absent from most mature ribosomes. The factor acts preferentially on the precursor form of 16S RNA and, in the conventional reconstitution system used here, does not seem to function catalytically, but to be used up by the assembly reaction.

We propose that in the cell a factor exists which has the role of lowering the energy of activation required to convert precursor particles into 30S subunits. Because of its higher affinity for p16S than for m16S RNA, the factor would be released from completed ribosomes only after rRNA is matured. rRNA maturation, on the other hand, probably occurs in polyribosomes (ref. 9 and G.M., E.T., C.P. and F.A., unpublished). Thus the recycling of the morphopoietic factor, and therefore the completion of nascent ribosomes, would be subordinated to the entry of previously formed particles into the pool of functioning ribosomes.

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Hybridisation of Dane particle DNA with the free plasma DNA of hepatitis carriers

PLASMA of hepatitis B patients and antigen carriers contains a high concentration of spherical and filamentous particles (HB_sAg), about 20 nm in diameter and without evidence of nucleic acid. There are also a relatively few spherical particles, 42 nm in diameter with a 28 nm core, first described by Dane *et al.*¹ Dane particles contain a unique deoxyribonucleic acid² and a DNA polymerase³ capable of synthesising DNA so they are considered to be a leading candidate for a human hepatitis B virus. Double stranded DNA of unique base composition has been isolated from the naked intranuclear particle of hepatocytes of human hepatitis liver⁴. The large quantity of HB_sAg, resembling defective virus, in the plasma of carriers prompted us to investigate a possibility that there may exist free or 'unassembled' DNA corresponding to the empty particles in these plasmas. The presence of both free DNA and empty particles could result from faulty assembly of a virus. We report here the presence of free DNA in HB_sAg positive plasma and this free DNA showed homology with radiolabelled Dane particle DNA by molecular hybridisation.

Plasmas from commercial donors were screened by radioimmunoassays for HB_sAg according to Ling *et al.*⁵ In a typical procedure for DNA isolation, about 50 ml of plasma were dialysed against Tris buffer (0.01 M Tris-HCl, pH 8.25; 0.1 M NaCl) containing 0.01% diethylpyrocabonate. Solid caesium chloride was added to the dialysed plasma to an average density of about 1.65, and the solution was then centrifuged in six buckets of a Spinco SW41 rotor at 35,000 r.p.m. for 67 h at 22° C. Because of the large amount of serum protein present in the sample, absorbance readings of the collected fractions were high and generally did not reveal separation of components. Occasionally, however, a small peak of absorbancy at 260 nm appeared above the high background around a density of 1.7

(Fig. 1a). Fractions around this density, regardless of the presence of an absorbancy peak or not, were combined and dialysed against the same buffer. One or two recentrifugations of the sample in CsCl revealed DNA, if present, at a density of 1.698. The absorbance profile of a final centrifugation from a DNA-positive sample is shown in Fig. 1b. The DNA showed a density of 1.423 in Cs₂SO₄, S_{20,w} values ranging from 10 to 22, and a T_m of 72° C in phosphate buffer (0.01 M, pH 7.15; 1 mM EDTA). The DNA appeared to be composed of linear double stranded molecules of about 2–3 μm. Details of the characterisation of this DNA will be described elsewhere.

Synthesis and isolation of ³H-thymidine labelled DNA from Dane particles were carried out as previously described³. Because of the minute quantity of DNA recovered from Dane particles, specific activities of the radiolabelled DNA could not be estimated. Hybridisation of the radioactive DNA and the plasma DNA was done according to a published method⁶ with the following modifications. About 2,500 c.p.m. of ³H-thymidine labelled DNA from Dane particles, and the non-radioactive

Table 1 Hybridisation between free DNA of plasma and ³H-DNA of Dane particles

Plasma	No. of samples	No. of DNA-positive sample	No. of DNA-positive sample hybridised	No. of positive hybridisation
HB _s Ag positive	21	21*	5	5†
HB _s Ag negative	21	2	2	2

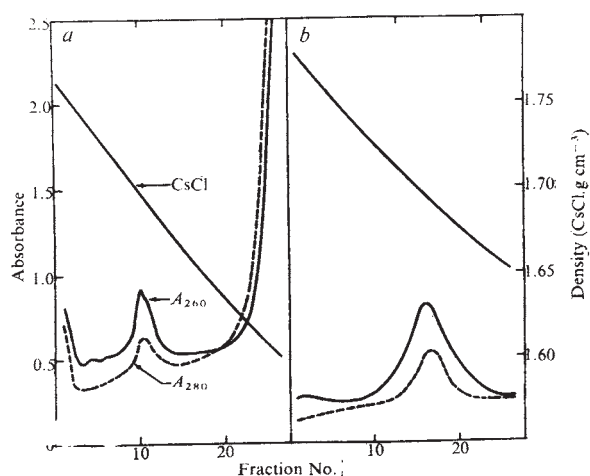
* In a DNA-positive sample, A₂₆₀ readings of the collected fractions from a 50 ml plasma were at least 0.01 above the background after the second CsCl centrifugation.

† In a positive hybridisation 1 μg of DNA protected more than 50% of the radio labelled Dane particle DNA from S1 nuclease digestion. Details of hybridisation conditions are described in the text.

plasma DNA were denatured, separately, with NaOH at pH 13 for 60 min at 37° C. Both DNA samples were then neutralised with HCl and incubated together in 1 × SSC in a total volume of 130 μl for 4 h at 68° C. The quantity of cold DNA varied from 0.1 to 1.0 μg, estimated from spectrophotometric absorption where 24 A₂₆₀ units were equivalent to 1 mg. After incubation, unhybridised, single-stranded DNA was digested with 1,000 units of S1 nuclease (Seikagaku Kogyo, Japan) for 2 h at 37° C. Calf thymus DNA (50 μg) was then added as a carrier and the reaction mixture was pipetted on 3 mm paper for washings in TCA according to Mans and Novelli⁷. Radioactivity was determined in a Beckman scintillation spectrometer. Negative controls in which cold DNA was omitted from hybridisation, served as the background of self-annealing which averaged about 10–15% of the input radioactivity. The value of 100% hybridisation was provided from reaction mixtures treated identically except that no S1 nuclease was used. DNA from human diploid cells (WI 38) and human liver tissue was isolated according to a published procedure⁸. Salmon sperm DNA and calf thymus DNA were purchased from the Sigma Chemical Company. These four DNA preparations were sonicated to give sizes comparable to plasma DNA with a S_{20,w} range of 10–25.

It was evident from the results of hybridisation (Fig. 2) that the DNA isolated from HB_sAg positive plasma by isopycnic banding in CsCl possessed homology with the radioactive Dane particle DNA. Similar results in hybridisation were obtained with DNA after further purification by phenol extraction and alcohol precipitation. DNA from human liver, WI 38 cells, salmon sperm and calf thymus showed no evidences of homology. In the experimental conditions used, the C₀t₁ of the plasma DNA was estimated to be about 9 × 10⁻² mol s l⁻¹. This value was considerably higher than that of a theoretical value of hybridisation (5 × 10⁻³) between Dane particle DNA having a molecular weight of 1.6 × 10⁶ with complexity of 2,600 nucleotide pairs¹⁰. This difference in observed and calculated C₀t₁ values could be due to two reasons. First, the hybridisation studies

Fig. 1 a, Isopycnic centrifugation of plasma in CsCl; b, the second CsCl centrifugation of the combined fractions around the density of 1.7 in a.



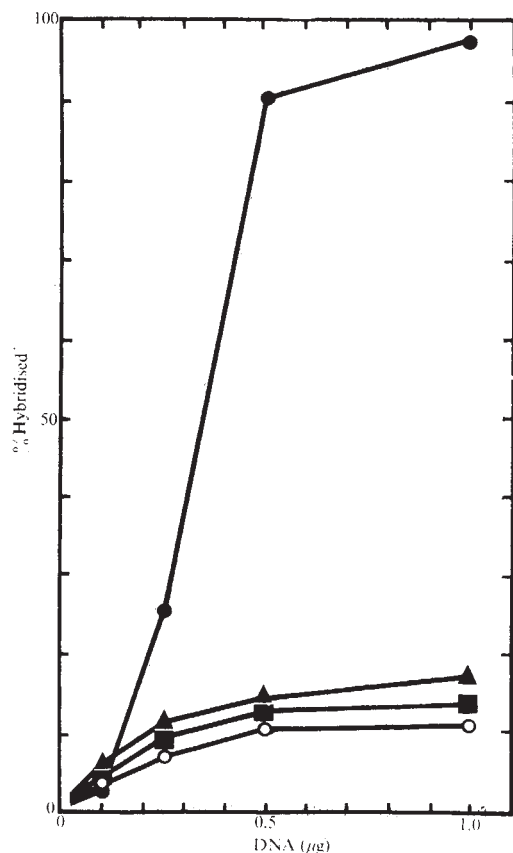


Fig. 2 Molecular hybridisation of plasma and heterologous DNA with ^3H -DNA of Dane particles. ●, HBsAg plasma DNA; ▲, salmon sperm DNA; ■, WI 38 DNA; ○, human liver DNA.

were conducted with molecules ($S_{20,w}$ of 10 to 22) much larger than small fragments of 400 to 500 nucleotides used to calculate the complexity of DNA from C_0t_1 values⁹. Thus the slower kinetics of reassociation with larger molecules would increase the C_0t_1 value¹⁰. Second, DNA not related to Dane particles, if present in the plasma DNA, would increase C_0t_1 . Further studies will be necessary to establish the full extent of homology between the DNA from plasma and the DNA produced by Dane particle polymerase.

Having found free DNA in a few plasmas from HBsAg carriers, we undertook a survey of 42 plasmas with regard to the presence or absence of free DNA and the ability of the DNA, if present, to hybridise with Dane particle DNA. The results of the study are shown in Table 1. In all of the 21 HBsAg positive plasmas examined, we found detectable amounts of free DNA with a recovered quantity equivalent to about $0.1\text{--}1\text{ }\mu\text{g ml}^{-1}$ of plasma. Five of these twenty-one DNA preparations were selected randomly and were found hybridisable to ^3H -DNA of Dane particles. In the group of HBsAg negative samples we found the majority of samples (19/21) negative in free DNA in that no detectable absorbance was found upon purifying the 1.7 density regions. Two of the samples, however, showed the presence of free DNA, and this DNA showed positive hybridisations with Dane particle DNA. The significance of this observation is not clear at this time, as we do not know whether free DNA in plasma is of host or viral origin. Further studies of these two plasmas are underway to assess fully the possible presence of HBsAg, undetectable with the methods employed.

The average length of the plasma DNA in electron micrographs was estimated to be $2\text{--}3\text{ }\mu\text{m}$, equivalent to a molecular weight of $4 \times 10^6\text{--}5 \times 10^6$. In some plasmas, the DNA concentration was the order of $1\text{ }\mu\text{g ml}^{-1}$, equivalent to 10^{10} molecules ml^{-1} . By comparison, a HBsAg concentration of $100\text{ }\mu\text{g ml}^{-1}$ in the plasma could give 3×10^{18} particles ml^{-1} , based on a

molecular weight of 2×10^6 daltons per particle. The length of DNA was longer than that of the circular genome of Dane particle DNA ($0.78\text{ }\mu\text{m}$). We have observed rolling circles in electron micrographs of the DNA isolated from Dane particles actively synthesising DNA (ref. 3). These rolling circles¹¹ with different lengths of linear segments would represent progeny DNA in polymeric forms. Although we found considerable homology between the DNA in plasma and in Dane particles, it is yet to be proven that the plasma DNA contains homology equivalent to one or more copies of the Dane particle genome.

The Dane particle is a leading candidate for the hepatitis B infectious agent, based on the findings that they contain a unique DNA primed with a DNA polymerase and are found in infectious plasmas of HBsAg carriers. In spite of the routine screening of blood donors with sensitive HBsAg immunological procedures, only about half of the cases of hepatitis B associated with blood transfusion seem to be related to the presence of HBsAg or antibody in donor blood, as revealed by these methods. The findings in this report on the presence of free DNA having homology to the DNA of Dane particles suggests the possibility of using molecular hybridisation in conjunction with immunological techniques for assessing potential infectivity of blood plasmas.

We thank Dr T. Kakefuda for electron microscopic studies of DNA, Dr W. Holleman for analytical centrifugation studies and W. Schulze for technical assistance.

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Errata

In the article "Flow near an oscillating cylinder in dilute viscoelastic fluid" by C. Chang and W. R. Schowalter (*Nature*, **252**, 686; 1974), Figs 2b and 3b should be interchanged.

In the article "Ambisomic reproduction of directionality in surround-sound systems" by P. B. Fellgett (*Nature*, **252**, 534; 1974) the following corrections are necessary. Page 537 line 6, for π read 2π ; line 9, for hyper-spheres read elliptic spaces; in ref. 3 for 189 read 1892 (date); in Fig. 2c the right-hand side of the CBS pan locus should be solid not dashed, to show that it is at the front (not the rear) of the sphere.

In the article by A. J. Cunningham (*Nature*, **252**, 749; 1974) the title should read "Large numbers of cells in normal mice produce antibody against components of isologous erythrocytes" and not as printed.

reviews

In January 1972 a study group of psychologists and ethologists met in London to discuss the growth of competence, under the aegis of the Developmental Sciences Trust and the Ciba Foundation. This book represents the fruits of their discussion; it comprises 16 papers presented either at the symposium or commissioned during its aftermath for inclusion in the published proceedings.

Most of the original papers have been revised in the light of comments made at the conference, and the editors have written introductory and concluding chapters. It is unusual, however, for such manoeuvres to transform a collection of individual papers on a common theme into a plausible book in which an identifiable argument is developed, and it must be said that *The Growth of Competence* has several of the characteristic flaws of the standard conference book. The most serious of these may be anticipated: the word 'competence' has such wide vernacular use that it comes as no surprise to discover between the book's covers almost as many definitions of its central concept as there are contributors. And even though this state of affairs may arise from the nature of the concept rather than from lexicographical laxness on the part of the authors, its

Competence in many forms

John N. Nicholson

The Growth of Competence. (Proceedings of a DST Study Group.) Edited by Kevin Connolly and Jerome Bruner. Pp. xii+327. (Academic: London and New York, September 1974.) £6.80; \$17.50.

effect on the book may be imagined. There are, moreover, a number of chapters which can with charity be described as lightweight. They neither include material interesting in its own right nor contribute to the argument which, in spite of the handicap mentioned above, is developed in the book; they are presumably included in deference to the book's function as a document of record.

These shortcomings are particularly regrettable in view of the book's praiseworthy intentions and the admirable quality of some of the contributions. It sets out to describe competence; to place it in an evolutionary context (a wide-ranging chapter by Bruner); to exemplify its development in a number of areas, including infants'

relationships with their mothers and their peers, skills (a detailed description of Elliott and Connolly's pioneering analysis of manual skills in normal and cerebral palsied children), language acquisition and personality; and to point out the practical applications of the experiments discussed and theoretical issues raised.

The book's central argument is carried forward by those contributors who follow Bruner in likening the growth of competence to the development of skills, which he regards as an hierarchically organised repertoire of sub-routines. This analogy is pushed furthest by Susan Carey in a provocative chapter on the development of cognitive competence, based on her penetrating reanalysis of the conservation problem. But in the end the book cannot escape the limitations of its genre. Most of the experimental work has been described elsewhere; it is unlikely therefore to become mandatory reading amongst developmental psychologists. Educationalists may welcome the discussion of the efficacy of schools as socialising agents in contemporary society, but they will find little cheer in the editors' pessimistic view of the contribution psychologists have thus far been able to make towards the solution of such problems. □

THE subject is fascinating. How is it that the principal area of technological rivalry between the two super-powers could within two years become the main field of cooperation in advanced projects? What shifts in foreign policy, in the scientific climate, even in personalities, mediated so radical a 'thaw'? Alas, one is not going to get more than a few hints from this book.

Was the untimely death of Dr Hugh Dryden, NASA's chief negotiator during the difficult early 1960s a significant setback? Did the 1964 Committee on Space Research (COSPAR) agreement on planetary contamination standards mark the first visible breakthrough of reason over rivalry (as was then widely reported)? There is no assessment of the first point and the second is not mentioned.

Disappointment with the book at first prompts the judgement that it is quite irrelevant. It is not; it is just biased. The book has apparently been developed through a series of studies by a team of authors drawing on a privileged

archive at the Center for Advanced International Studies of the University of Miami (CIAS). It presents a digest of official, documentary evidence and American reportage (which are given equal validity) within the matrix of the authors' own commentary.

The whole thing is set rolling in a predetermined direction with a 28-page

Detente in orbit

Angela Croome

U.S.-Soviet Cooperation in Space. By Dodd L. Harvey and Linda C. Ciccoritti. Pp. 350. (University of Miami: Florida, 1974.) \$8.95, paper; \$12.95, boards.

foreword by the United States Ambassador to Moscow in the mid-1960s, Foy D. Kohler, who is now luminary of CIAS. He discounts any possibility of an independent role or influence from Soviet scientists. He sees the entire pattern of cooperation as determined by Soviet political pressures. He may well be right but his own professional

allegiance reduces any conviction and the material adduced suggests that there has been some careful selection to support his thesis. Nor does he acknowledge the equally overriding influence of American political postures on the turn of events during the 15-year period leading up to the 1972 Nixon-Brezhnev Agreement (the space cooperation programme was, significantly, a part of the overall detente agreement).

It is useful to have chapter and verse of the Dean Rusk papers (1962) which concluded that space had no military value for the United States and which suggested that the Soviet Union should appreciate this before adopting counter measures to a non-existent threat. That was a turning point.

The volume's 100-page bibliography would be invaluable if it could be consulted independently of the text. As it is, this 'analysis' is journalistic in approach but does not have the convenience of having been written by journalists. □

Magnetic models

Experiments on Simple Magnetic Model Systems: A Survey of their Experimental Status in the Light of Current Theories. (Taylor and Francis Monographs on Physics.) By L. J. de Jongh and A. R. Miedema. Pp. 269. (Taylor and Francis: London, August 1974.) £4.00.

FORTY years elapsed between the discovery of superconductivity and the advent of a theoretical model describing the effect. A much greater period had passed at around the turn of the century when Weiss introduced the first theoretical model to account for another cooperative phenomenon, ferromagnetism. Since that time, increasingly complex and subtle models have been developed to allow more accurate calculations of thermodynamic quantities near phase transitions. The simplest models, restricted to one or two-dimensional crystal lattices, enable exact calculations and may seem to provide merely a playground for the theoretician. But those models now have, in fact, a relevance to the real world. Advancing technology has facilitated the identification and preparation of single crystals containing chains or layers of paramagnetic ions, isolated by distances of the order of tens of Angstroms, which, therefore, have a magnetic quasidimensionality of one or two.

A large part of this book is devoted to a review of the measured properties of such compounds and, although it is not easy reading, it provides an invaluable, and what must be an almost exhaustive, reference catalogue of these materials—the index to substances contains approximately 200 entries. The fitting of theory to experiment by judicious choice of the exchange constant is impressive in many cases.

There remain, however, some areas in which the available experimental analogues do not yet approximate closely enough to the models to confirm some theoretical predictions. An example of this is the two dimensional Heisenberg model, in which long-range order must be absent at finite temperatures: the expansion approach of the high temperature series suggests that the susceptibility will diverge at a finite temperature. The problem is resolved by identifying this temperature not with the normal phase transition but as a transition (the Stanley-Kaplan transition) to a state of infinite initial susceptibility with zero spontaneous magnetisation. Real materials deviate from the ideal because of the presence of anisotropy from single ion or dipolar contributions. Competing anisotropies can, however, be made to cancel, al-

though usually at a single temperature only. The best approach, therefore, seems to be to use a series of compounds in which the varying degree of deviation can be determined, then to extrapolate to the ideal. The authors believe that the evidence so obtained suggests that Stanley-Kaplan temperatures may exist.

In a final section a number of special topics are considered, including neutron diffraction and spin-wave theory.

This book, a reprint of *Advances in Physics*, 23, contains an elegant and concise review of the properties of the theoretical models of magnetic systems, and a comprehensive body of experimental data relevant to those models. As such, it is a useful addition to the library of the research worker in solid state magnetism.

W. O'Reilly

Insect behaviour

Experimental Analysis of Insect Behaviour. Edited by L. Barton Browne. Pp. viii+366. (Springer: Heidelberg, Berlin, New York, 1974.) \$15.40.

THIS volume originates from a symposium entitled "Experimental Analysis of Insect Behaviour" which formed part of the 14th International Congress of Entomology held in Canberra in 1972. The 25 contributions to the book include several not actually delivered at the symposium and some that are modified versions of the original papers. Most are written in the form of reviews, some of which are speculative,

emphasising particular areas of research, and some of which are of a more general nature.

Appropriately, because he addressed the closing plenary session of the Congress on "The emergence of behaviour", J. S. Kennedy's paper—"Changes of responsiveness in the patterning of behavioural sequences"—sets the tone of the volume by summarising his interpretation of flight and settling responses in *Aphis fabae*. The final paper, by Dingle, also deals with insect flight in the context of "The experimental analysis of migration and life history strategies in insects" and it is refreshing and encouraging to find many ecological concepts aired in such physiological company. Topics included in the intervening 23 papers include reproductive behaviour, feeding, several aspects of the insect nervous system and neural mechanisms, and hormone-mediated behaviour. Many of the papers are useful appraisals of recent work and concepts in the experimental analysis of insect behaviour, and it is convenient to have them so readily available.

Unfortunately, the editor has made little attempt to arrange the papers to present and develop an underlying theme, or to summarise the forward-looking approach that the symposium was, presumably, convened to stimulate. Indeed, the volume—reproduced from typescript—can only be considered as a 'book' in so far as it is bound between boards.

T. Lewis



Burial tombs of Tasmanian aborigines. From *Biogeography and Ecology in Tasmania*. (Monographiae Biologicae, vol. 25.) By W. D. Williams. Pp. 498+122 figs. (Junk: The Hague, 1974.) Dfl 140.

Physical Chemistry: An Advanced Treatise. Vol. VIA: *Kinetics of Gas Reactions*. Edited by William Jost. Pp. xx+507. (Academic: New York and London, November 1974.) \$43.00; £20.25.

THE editors of this series note in the Foreword that, because of the tremendous expansion in the development of techniques and principles of physical chemistry in recent years, most physical chemists "find it difficult to maintain an understanding of the entire field". This surely understates the problem, and I must admit, albeit sadly, that I find this difficulty even when restricted to my own chosen field of physical chemistry—kinetics—let alone the "entire field".

An advanced treatise should assume that the reader has at least a first degree level of knowledge, and this is certainly true of this volume. Indeed in some cases rather more is assumed of the reader.

The first chapter, on formal kinetics, by W. Jost makes an excellent start with a concise but clear survey of classical reaction kinetics, and leads on to a consideration of steady states, stability and, finally such topics as oscillating reactions; these latter topics have attracted much attention in the past decade. The next, short chapter, by C. F. Curtiss—A Survey of Kinetic Theory—is, in my opinion, too limited in scope and is at a level which surely assumes too much of a graduate student. It does not fit in happily with the rest of the volume. In the third chapter by H. Eyring and S. H. Lin, on potential energy surfaces, an account of the valence bond method is given and then surfaces for $H+H_2$, $Cl+Cl_2$ and $K+NaCl$ are considered in some detail. A final section on orbital symmetry in reaction kinetics does not blend well with the rest of the chapter and deserves a chapter of its own.

Chapter 4 is again short—E. E. Nikitin on the Theory of Energy Transfer in Molecular Collisions. The translation, though clear, is a little stilted and the treatment is too condensed; few readers will find it an easy introduction to this important topic. The final chapters constitute more than half this volume. Chapter 5 by J. P. Toennies is on molecular beam scattering experiments and considers elastic, inelastic and reactive collisions. This is a field that is progressing rapidly and this chapter presents a balanced and clear account of it. Chapter 6 by J. C. Polanyi and J. L. Schreiber on the dynamics of bimolecular reactions must, of necessity, cover some of the same ground as the earlier chapters. Such duplication as there is, however, is not serious, and the viewpoint is

somewhat different. Again, this chapter presents a clear account which most graduate students interested in kinetics will find valuable.

The chapter headings to the companion volume (VIB) on gas kinetics suggest that the two will fit together well. It is, however, sad to note how many important areas of gas kinetics will not be covered at all.

This book can be recommended for libraries, but at £20.25 it is hardly cheap and is unlikely to find itself on the shelves of all those 'physical chemists' for whom it was, no doubt, intended.

H. M. Frey

Gas works

Theory of Elementary Atomic and Molecular Processes in Gases. By E. E. Nikitin. Translated by M. J. Kearsley. Pp. xiii+472. (Clarendon: Oxford; Oxford University Press: London, August 1974.) £14.75.

THE author of this book is one of the world's leading authorities on the theory of rate processes, who has extensive and detailed research experience in many branches of the subject. This translation from the Russian version originally published in 1970 is, therefore, more than welcome. The emphasis rests on the theory of processes relevant to the chemical reactions discussed. The main body of the book makes reference to the literature published before the end of 1966, with an added appendix that includes brief reference to developments in the two or three years following that date.

Professor Nikitin presents a very wide, well integrated view of the subject as a whole, and thus the material in the various chapters of the book is well organised, with chapters following one another in a smooth fashion. The first chapter on the transition state method is presented in a very formal manner, going far beyond the statistical mechanical treatment normally found in standard books on kinetics. The following chapter, dealing with the transfer of translational motion to other forms of energy, especially to vibration, for which there is a large body of experimental data, and also to electronic energy in both atoms and diatomic molecules, is extensive and highly authoritative.

The chapter formally entitled "Unimolecular Reactions" may seem to be relatively limited, considering the scope of the subject, but it is a tight and formal approach. It follows well the chapter concerned with vibrational energy transfer and includes a section on surface crossing and "non-adiabatic

transitions". With that chapter one should also consider the following detailed treatment of the statistical theory of reactions. Similarly, the presentation of the diffusion theory of reactions, which regards chemical reactions as diffusion processes of representative points in phase space from regions corresponding to reactants to regions corresponding to products, is also highly relevant to the considerations of unimolecular reactions given in earlier chapters.

The dissociation of diatomic molecules and the reverse process of atomic recombination are dealt with more in terms of the types of results obtained from different theories than in terms of detailed developments of the leading theories. The appeal to experiment is very limited but the chapter is rich in the physical principles at issue in such processes. The basis of semi-empirical methods for bimolecular reactions is presented with particular clarity, and leads in due course to the more recent, *ab initio* calculations of Bruner and Conroy for the H_2 system.

Direct reactions and the dynamics of exchange reactions, of particular relevance to processes studied in molecular beams, are also dealt with fully. The appendix, clearly written after the main body of the book, is a useful brief resumé of the objectives of theories of chemical reactions. As the book is designed for post graduate chemists and physicists, the appendix could usefully be read first, especially as there is no general introduction.

Since the completion of the book, there has been a major development in the experimental study of many fundamental aspects of atomic reactions. Work on the collisional behaviour of atoms in specific electronic states, and various studies aimed towards the development of a framework within which context the relationship between electronic structure and atomic reactivity can be understood, comprise much of this development. These areas of research occupy little space, however, and it is to be hoped that the next edition will contain much more of that type of work, including Professor Nikitin's own theoretical researches.

For research workers concerned either with the theory or experiment of fundamental rate processes in gases, access to this book is barely a matter of choice but rather of basic necessity. Occasionally, the price of a book must be judged not only in relation to its size but to the quality of its content. This is such a book; it is hard to imagine a library without it, and it is to be hoped that research workers will consider that a classic work of this kind merits a place on their personal bookshelves.

D. Husain

obituary

Francis J. Weiss, the biochemist and economist concerned with developing food resources, died on January 21, at the age of 76.

Dr Weiss was born in Vienna, receiving doctorates in chemistry and economic statistics at the University there. He worked in the biochemistry institute of the University of Vienna until the Nazis occupied Austria in 1938, at which time he left for England. A year later, he went to the United States, where he was a consultant to the Board of Economic Warfare and the Sugar Research Foundation. In the fifties, he was a consultant on the staff of Senator Hubert H. Humphrey, studying chemical agriculture and new methods of processing grains. Later, he was a consultant to the International Cooperation Administration and the Department of Commerce. From 1959 to 1969, he was a specialist in the science and technology division of the Library of Congress.

Vladimir Aleksandrovich Fock, academician, and one of the leading theoretical physicists in the Soviet Union, died on December 27, 1974, five days after his 76th birthday.

Fock, who graduated from the University of Petrograd (now Lenin-

grad) in 1922, throughout his working life remained connected with this University, teaching and researching there. He also at various times worked at the State Institute of Optics, the Leningrad Institute of Physics and Technology, and the Institute of Physics of the Soviet Academy of Sciences. In 1932 he was elected a Corresponding Member, and in 1939 a full member of the Soviet Academy of Sciences. Fock's basic research was concerned with quantum mechanics, quantum electrodynamics, electromagnetic diffraction and radiowave propagation, the general theory of relativity, mathematics, and mathematical physics, in all of which fields he established new basic concepts. He is particularly associated with the establishment of the scalar relativistic wave equation for a particle with no spin in an electromagnetic field, which he derived independently of similar work by the Swedish physicist O. Klein (the 'Klein-Fock equation'). His name is also associated with spaces of an increasing number of dimensions ('Fock spaces'), which he used to obtain a quantum description of systems with a variable number of Bose particles. For his services to Soviet science, Fock was awarded the Order of Lenin (four

times), a Stalin Prize (now State Prize) in 1946 and a Lenin Prize in 1960. He was also a member of a number of foreign and international academies and learned societies.

Matthew W. Stirling, the distinguished archaeologist, died on January 23, at the age of 78.

An anthropologist and archaeologist associated with the Smithsonian Institution for more than 40 yr, Dr Stirling travelled widely in Central and South America, discovering "America's oldest dated work"—a stone monument bearing a date equivalent to 291 BC. He has headed many important expeditions in the Americas and in Europe, but will be known primarily for his contributions to middle American anthropology and archaeology. Under the auspices of both the Smithsonian and the National Geographic Society, Dr Stirling led the team of archaeologists who uncovered the La Venta, or Olmec, civilisation dating back more than 1,000 yr. Dr Stirling was born in California and graduated from the University there in 1920. He was affiliated with the Smithsonian from 1921 until his retirement in 1958. He also served as head of its bureau of American Ethnology from 1928 to 1947.

announcements

International meetings

March 13-14, **Global Tectonics in Proterozoic Times**, London (Executive Secretary, Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

April 20-23, **Cybernetics and Systems Research**, Vienna (Secretariat of the Austrian Society for Cybernetic Studies, Schottengasse 3, A-1010 Wien, Austria).

May 6, **Crop Protection**, Gent, Belgium (The Secretary, Faculty of Agricultural Sciences, State University, Coupure links, 533, B-9000 Gent, Belgium).

Reports and publications

Great Britain

Proceedings of the Royal Irish Academy. Vol. 75, Section A, No. 14: Shrinking and Boundedly Complete Bases of Projections. By J. J. M. Chadwick. Pp. 95-102, 38p. Vol. 74, Section B, No. 18: Biology of the Rudd *Scardinius erythrophthalmus* (L.) in Irish Waters.

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